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(21) International Application Number: PCT/US95/15516 (22) International Filing Date: 22 November 1995 (22.11.95)			(72) Inventors: BEIGELMAN, Leonid; 5530 Colt Drive, Longmont, CO 80503 (US). STINCHCOMB, Daniel, T.; 7203 Old Post Road, Boulder, CO 80301 (US). JARVIS, Thale; 2925 Glenwood Drive #301, Boulder, CO 80301 (US). DRAPER, Kenneth; 4619 Cloud Court, Boulder, CO 80301 (US). PAVCO, Pamela; 4619 Cloud Court, Boulder, CO 80301 (US). MCSWIGGEN, James; 4866 Franklin Drive, Boulder, CO 80301 (US). GUSTOFSON, John; 4866 Franklin Drive, Boulder, CO 80301 (US). USMAN, Nassim; 2954 Kalmia #37, Boulder, CO 80304 (US). WINCOTT, Francine; 7920 N. 95th Street, Longmont, CO 80501 (US). MATULIC-ADAMIC, Jasenka; 760 South 42nd Street, Boulder, CO 80303 (US). KARPEISKY, Alexander; 5121 Williams Fork Trail #209, Boulder, CO 80301 (US). THOMPSON, James, D.; 2925 Glenwood Drive #301, Boulder, CO 80301 (US). MODAK, Anil; 3855 Hauptman Court, Boulder, CO 80301 (US). BURGIN, Alex; 3115 Gatling Lane, Boulder, CO 80301 (US). (74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon, First Interstate World Center, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US). (81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>Without international search report and to be republished upon receipt of that report.</i>																																
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(54) Title: METHOD AND REAGENT FOR TREATMENT OF ARTHRITIC CONDITIONS, INDUCTION OF GRAFT TOLERANCE AND REVERSAL OF IMMUNE RESPONSES (57) Abstract An enzymatic nucleic acid molecule which cleaves RNA associated with development or maintenance of an arthritic condition, induction of graft tolerance or reversal of an immune response. In particular, the ribozyme sequences are directed to an mRNA encoding B7-1, B7-2, B7-3, CD40 and/or stromelysin. Also provided are ribozymes where the uracil in positions 4 and/or 7 are substituted, as well as methods for the synthesis of 2'-alkylnucleotides, 2'-O-alkylthioalkyl, or 2'-alkylthioalkylnucleotides. The application further describes a method for diprotection of RNA with aqueous ethylamine, a method for synthesis of a basic ribonucleoside mimetics, and transcription units comprising an RNA polymerase II promoter, a U6 small nuclear promoter, or an adenovirus VA1 promoter system.																																			

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METHOD AND REAGENT FOR TREATMENT OF ARTHRITIC
CONDITIONS. INDUCTION OF GRAFT TOLERANCE AND
REVERSAL OF IMMUNE RESPONSES

5

Background of the Invention

The following is a discussion of relevant art, none of which is admitted to be prior art to the present invention.

10 In one aspect, this invention relates to methods for inhibition of osteoarthritis, in particular, inhibition of genetic expression which leads to a reduction or elimination of extracellular matrix digestion by matrix metalloproteinases.

15 There are several types of arthritis, with osteoarthritis and rheumatoid arthritis being predominant. Osteoarthritis is a slowly progressive disease characterized by degeneration of articular cartilage with proliferation and remodeling of subchondral bone. It presents with a clinical picture of pain, deformity, and loss of joint motion. Rheumatoid arthritis is a chronic systemic inflammatory disease. Rheumatoid arthritis may be mild and relapsing or severe and progressive, leading to joint deformity and incapacitation.

20 Arthritis is the major contributor to functional impairment among the older population. It is the major cause of disability and accounts for a large proportion of the hospitalizations and health care expenditures of the elderly. Arthritis is estimated to be the principal cause of total incapacitation for about one million persons aged 55 and older and is thought to be an important contributing cause for about one million more.

25 Estimating the incidence of osteoarthritis is difficult for several reasons. First, osteoarthritis is diagnosed objectively on the basis of reading radiographs, but many people with radiologic evidence of disease have no obvious symptoms. Second, the estimates of prevalence are based upon clinical evaluations because radiographic data is not available for all afflicted joints. In the NHANESI survey of 1989, data were based upon a thorough musculoskeletal evaluation during which any abnormalities of the spine, knee,

30

hips, and peripheral joints were noted as well as other specific diagnoses. Based on these observations, 12% of the US population between 25 and 74 years of age have osteoarthritis.

5 It is generally agreed that rheumatoid arthritis has a world-wide distribution and affects all racial and ethnic groups. The exact prevalence in the US is unknown but has been estimated to range between 0.5% and 1.5%. Rheumatoid arthritis occurs at all age levels and generally increases in prevalence with advancing age. It is 2-3 times more prevalent in women than in men and peak incidence occurs between 40-60 years of age. In addition to
10 immunological factors, environmental, occupational and psychosocial factors have been studied for potential etiologic roles in the disease.

The extracellular matrix of multicellular organisms plays an important role in the formation and maintenance of tissues. The meshwork of the extracellular matrix is deposited by resident cells and provides a framework for
15 cell adhesion and migration, as well as a permeability barrier in cell-cell communication. Connective tissue turnover during normal growth and development or under pathological conditions is thought to be mediated by a family of neutral metalloproteinases, which are zinc-containing enzymes that require calcium for full activity. The regulation of metalloproteinase
20 expression is cell-type specific and may vary among species.

The best characterized of the matrix metalloproteinases, interstitial collagenase (MMP-1), is specific for collagen types I, II, and III. MMP-1 cleaves all three chains of the triple helix at a single point initiating sequential
25 breakdown of the interstitial collagens. Interstitial collagenase activity has been observed in rheumatoid synovial cells as well as in the synovial fluid of patients with inflammatory arthritis. Gelatinases (MMP-2) represent a subgroup of the metalloproteinases consisting of two distinct gene products; a 70 kDa gelatinase expressed by most connective tissue cells, and a 92 kDa gelatinase expressed by inflammatory phagocytes and tumor cells. The larger
30 enzyme is expressed by macrophages, SV-40 transformed fibroblasts, and neutrophils. The smaller enzyme is secreted by H-ras transformed bronchial epithelial cells and tumor cells, as well as normal human skin fibroblasts. These enzymes degrade gelatin (denatured collagen) as well as native

collagen type XI. Stromelysin (MMP-3) has a wide spectrum of action on molecules composing the extracellular matrix. It digests proteoglycans, fibronectin, laminin, type IV and IX collagens and gelatin, and can remove the N-terminal propeptide region from procollagen, thus activating the collagenase. It has been found in human cartilage extracts, rheumatoid synovial cells, and in the synovium and chondrocytes of joints in rats with collagen-induced arthritis.

Both osteoarthritis and rheumatoid arthritis are treated mainly with compounds that inhibit cytokine or growth-factor induced synthesis of the matrix metalloproteinases which are involved in the extracellular matrix destruction observed in these diseases. Current clinical treatments rely upon dexamethasone and retinoid compounds, which are potent suppressors of a variety of metalloproteinases. The global effects of dexamethasone and retinoid treatment upon gene expression in treated cells make the development of alternative therapies desirable, especially for long term treatments. Recently, it was shown that gamma-interferon suppressed lipopolysaccharide induced collagenase and stromelysin production in cultured macrophages. Also, tissue growth factor- β (TGF- β) has been shown to block epidermal growth factor (EGF) induction of stromelysin synthesis in vitro. Experimental protocols involving gene therapy approaches include the controlled expression of the metalloproteinase inhibitors TIMP-1 and TIMP-2. Of the latter three approaches, only γ -interferon treatment is currently feasible in a clinical application.

Sullivan and Draper, International PCT Publication No. WO 94/02595 and Draper *et al.*, International PCT Publication No. WO 95/13380 disclose the use of ribozymes to treat arthritis.

In a second aspect, the invention relates to methods for the induction of graft tolerance, treatment of autoimmune diseases, inflammatory disorders and allergies in particular, by inhibition of B7-1, B7-2, B7-3 and CD40.

An adaptive immune response requires activation, clonal expansion, and differentiation of a class of cells termed T lymphocytes (T cells). T cell activation is a multi-step process requiring several signalling events between

the T cell and an antigen presenting cell. The ensuing discussion details signals that are exchanged between T cells and antigen presenting B cells. Similar pathways are thought to occur between T cells and other antigen presenting cells such as monocytes or follicular dendritic cells.

5 T cell activation is initiated when the T-cell receptor (TCR) binds to a specific antigen that is associated with the MHC proteins on the surface of an antigen presenting cell. This primary stimulus activates the T cell and induces expression of CD40 ligand (CD40L) on the surface of the T cell. CD40L then
10 interacts with its cognate receptor, CD40, which is constitutively expressed on the surface of B cells; CD40 transduces the signal leading to B cell activation. B cell activations result in the expression of B7-1, B7-2 and/or B7-3, which in turn interacts with constitutively expressed CD28 on the surface of T cells. The interaction generates a secondary co-stimulatory signal that is required to fully activate the T cell. Complete T cell activation via the T cell receptor and CD28
15 leads to cytokine secretion, clonal expansion, and differentiation. If the T cell receptor is engaged, absence of this secondary co-stimulus mediated by CD28, then the T cell is inactivated, either by clonal anergy (non-responsiveness or reduced reactivity of the immune system to specific antigen(s)) or clonal deletion (Jenkins et al., 1987 *Proc. Natl. Acad. Sci. USA*
20 84, 5409). Thus, engagement of the TCR without a concomitant costimulatory signal results in a state of tolerance toward the specific antigen recognized by the T cell. This co-stimulatory signal can be mediated by the binding of B7-1 or B7-2 or B7-3, present on activated antigen-presenting cells, to CD28, a receptor that is constitutively expressed on the surface of the T cell
25 (Marshall et al., 1993 *J Clin Immun* 13, 165-174; Linsley, et al., 1991 *J Exp Med* 173, 721; Koulova et al., 1991 *J Exp Med* 173, 759; Harding et al., 1992 *Nature* 356, 607).

Several homologs of B7 (now known as B7-1; Cohen, 1993 *Science*
262, 844) are expressed in activated B cells (Freeman et al., 1993 *Science*
30 262, 907; Lenschow et al., 1993 *Proc Natl Acad Sci USA* 90, 11054; Azuma et al., 1993 *Nature* 366, 76; Hathcock et al., 1993 *Science* 262, 905; Freeman et al., 1993 *Science* 262, 909). B7-1 and B7-3 are only expressed on the surface of a subset of B cells after 48 hours of contact with T cells. In contrast, B7-2 mRNA is constitutively expressed by unstimulated B cells and increases 4-fold

within 4 hours of activation (Freeman et al., 1993 *Science* 262, 909; Boussiotis et al., 1993 *Proc Natl Acad Sci USA* 90, 11059). Since T cells commit to either the anergy or the activation pathway within 12-24 hours of the initial TCR signal, it is thought that B7-2 is the molecule responsible for the primary
5 costimulatory signal. B7-1 and B7-3 may provide a subsequent signal necessary for clonal expansion. Antibodies to B7-2 completely block T cell proliferation in a mixed lymphocyte reaction (Azuma et al., 1993 *supra*), supporting the central role of B7-2 in T cell activation. These experiments indicate that inhibition of B7-2 expression (for example with a ribozyme) would
10 likely induce anergy. Similarly, inhibition of CD40 expression by a ribozyme would prevent B7-2 upregulation and could induce tolerance to specific antigens.

B7 (B7-1) is a 60 KD modified trans-membrane glycoprotein usually present on the surface of antigen presenting cells (APC). B7 has two ligands—
15 CD28 and CTLA4. Interaction of B7-1 with CD28 and/or CTLA4 causes activation of T cell responses (Janeway and Bottomly, 1994 *Cell* 76, 275).

B7-2 is a 70 KD (34 KD unmodified) trans-membrane glycoprotein found on the surface of APCs. B7-2 encodes a 323 amino-acid protein which is 26
20 % identical to human B7-1 protein. Like B7-1, CD28 and CTLA4 are selectively bound by B7-2. B7-2, unlike B7-1, is expressed on the surface of unstimulated B cells (Freeman et al., 1993 *supra*).

CD40 is a 45-50 KD surface glycoprotein found on the surface of late pre-B cells in bone marrow, mature B cells, bone marrow-derived dendritic cells and follicular dendritic cells (Clark and Ledbetter, 1994 *Nature* 367, 425).

25 Successful organ transplantation currently requires suppression of the recipient's immune system in order to prevent graft rejection and maintain good graft function. The available therapies, including cyclosporin A, FK506 and various monoclonal antibodies, all have serious side effects (Caine, 1992 *Transplantation Proceedings* 24, 1260; Fuleihan et al., 1994 *J. Clin. Invest.* 93,
30 1315; Van Gool et al., 1994 *Blood* 83, 176). In addition, existing therapies result in general immune suppression, leaving the patient susceptible to a variety of opportunistic infections. The ability to induce a state of long-term,

antigen-specific tolerance to the donor tissue would revolutionize the field of organ and tissue transplantation. Since organ graft rejection is mediated by T cell effector function, the goal is to block specifically the activation of the subset of T cells that recognize donor antigens. A limitation in the field of
5 transplantation is the supply of donor organs (Nowak 1994 *Science* 266, 1148). The ability to induce donor-specific tolerance would substantially increase the chances of successful allografts, xenografts, thereby greatly increasing the donor pool.

Such transplantation includes grafting of tissues and/or organ i.e.,
10 implantation or transplantation of tissue and/or organs, from the body of an individual to a different place within the same or different individual. Transplantation also involve grafting of tissues and/or organs from one area of the body to another. Transplantation of tissues and/or organs between genetically dissimilar animals of the same species is termed as allogeneic
15 transplantation. Transplantation of animal organs into humans is termed xenotransplants (for a review see Nowak, 1994 *Science* 266, 1148).

One therapy currently being developed that has similar potential to induce antigen-specific tolerance is treatment with a CTLA4-Ig fusion protein. "CTLA4" is a homologue of CD28 that binds B7-1 and B7-2 with high affinity.
20 The engineered, soluble fusion protein, CTLA4-Ig, binds B7-1, thereby blocking its interaction with CD28. The results of CTLA4-Ig treatment in animal studies are mixed. CTLA4-Ig treatment significantly enhanced survival rates and ameliorated the symptoms of graft-versus host disease in a murine bone marrow transplant model (Blazer et al., 1994 *Blood* 83, 3815). CTLA4-Ig
25 induced long-term (>110 days) donor-specific tolerance in pancreatic islet xenografts (Lenschow et al., 1992 *Science* 257, 789). Conversely, in another study CTLA4-Ig treatment delayed but did not ultimately prevent cardiac allograft rejection (Turka, et al., 1992 *Proc Natl Acad Sci U S A* 89, 11102). Mice immunized with sheep erythrocytes in the presence of CTLA4-Ig failed to
30 mount a primary immune response (Linsley, et al., 1992 *Science* 257, 792). A secondary immunization did elicit some response, however, indicating incomplete tolerance. Interestingly, identical results were obtained when CTLA4-Ig was administered 2 days after primary immunization, leading the authors to conclude that CTLA4-Ig blocked amplification rather than initiation

of the immune response. Since CTLA4-Ig has been shown to dissociate more rapidly from B7-2 compared with B7-1, this may explain the failure to induce long term tolerance in this model (Linsley et al., 1994 *Immunity* 1, 793).

5 CTLA4:Ig has recently been shown to ameliorate symptoms of spontaneous autoimmune disease in lupus-prone mice (Finck et al., 1994 *Science* 265, 1225).

Linsley et al., WO 92/00092 describe B7 antigen as a ligand for CD28 receptor on T cells. The application states that—

10 "The B7 antigen, or its fragments or derivatives are reacted with CD28 positive T cells to regulate T cell interactions with other cells..... B7 antigen or CD28 receptor may be used to inhibit interaction of cells associated with these molecules, thereby regulating T cell responses."

De Boer and Conroy, WO 94/01547 describe the use of anti-B7 and anti-CD40 antibodies to treat allograft transplant rejection, graft versus host disease and rheumatoid arthritis. The application states that—

15 "...anti-B7 and anti-CD40 antibodies...can be used to prevent or treat an antibody-mediated or immune system disease in a patient."

Since signalling via CD40 precedes induction of B-7, blocking the CD40-CD40L interaction would also have the potential to produce tolerance. According to one report, simultaneous treatment of mice with antibodies to
20 CD40L and sheep red blood cells produced antigen-specific tolerance for up to 3 weeks following cessation of treatment (Foy et al., 1993 *J Exp Med* 178, 1567). Anti-CD40L also produces antigen specific tolerance in a pancreatic islet transplant model (R. Noelle, personal communication). Targeted inhibition of CD40 expression in B cells in addition to B7 would therefore
25 afford double protection against activation of T cells.

Therapeutic agents used to prevent rejection of a transplanted organ are all cytotoxic compounds or antibodies designed to suppress the cell-mediated immune system. The side effects of these agents are those of immunosuppression and infections. The primary approved agents are
30 azathioprine, corticosteroids, cyclosporine; the antibodies are antilymphocyte or antithymocyte globulins. All of these are given to individuals who have been as closely matched as possible to their donors by both major and minor

histocompatibility typing. Since the principal problem in transplantation is an antigenic mismatch and the resulting need for cytotoxic therapy, any therapeutic improvement which decreases the local immune response without general immunosuppression should capture the transplant market.

5 Cyclosporine: At the end of the 1970's and early 1980's the introduction of cyclosporine revolutionized the transplantation field. It is a potent immunosuppressant which can inhibit immunocompetent lymphocytes specifically and reversibly. Its primary mechanism of action appears to be inhibition of the production and release of interleukin-2 by T helper cells. In
10 addition it also interferes with the release of interleukin-1 by macrophages, as well as proliferation of B lymphocytes. It was approved by the FDA in 1983 and by 1989 was almost universally given to transplant recipients. At first it was believed that the toxicity and side effects from cyclosporine were minimal and it was hailed as a "wonder drug." Numerous side effects have been
15 progressively cited, including the appearance of lymphomas, especially in the gastrointestinal tract; acute and chronic nephrotoxicity; hypertension; hepatotoxicity; hirsutism; anemia; neurotoxicity; endocrine and neurological complications; and gastrointestinal distress. It is now widely acknowledged that the non-specific side effects of the drug demand caution and close
20 monitoring of its use. One-year survival rates for cadaver kidney transplants treated with cyclosporine is 80%, much better than the 50-60% rates without the drug. The one-year survival is almost 90% for transplants with related donors and the use of cyclosporine.

25 Azathioprine: In addition to cyclosporine, azathioprine is used for transplant patients. Azathioprine is one of the mercaptopurine class of drugs and inhibits nucleic acid synthesis. Patients are maintained indefinitely on daily doses of 1mg/kg or less, with a dosage adjusted in accordance with the white cell count. The drug may cause depression of bone marrow elements and may cause jaundice.

30 Corticosteroids: Prednisone, used in almost all transplant recipients, is usually given in association with azathioprine and cyclosporine. The dosage must be regulated carefully so as to prevent complications such as infection, development of cushingoid features, and hypertension. Usually the initial

maintenance prednisone dosage is 0.5 mg/kg/d. This dosage is usually further decreased in the outpatient clinic until maintenance levels of about 10 mg/d for adults are obtained. The exact site of action of corticosteroids on the immune response is not known.

- 5 Antithymoblast or antilymphocyte globulin (ALG) and antithymocyte globulin (ATG): These are important adjunctive immunosuppressants. They are effective, particularly in induction of immunosuppressive therapy and in the treatment of corticosteroid-resistant rejection. Both ALG and ATG can be made by immunizing horses, rabbits, or sheep; the main source is horses.
- 10 Lymphocytes from human peripheral blood, spleen, lymph nodes, or thymus serve as the immunogen.

- Tacrolimus: On April 13, 1994 the Food and Drug Administration approved another drug to help prevent the rejection of organ transplants. The drug, tacrolimus, was approved only for use in liver transplant patients. An
- 15 alternative to cyclosporine, the macrolide immunosuppressant tacrolimus is a powerful and selective anti-T-lymphocyte agent that was discovered in 1984. Tacrolimus, isolated from the fungus *Streptomyces tsukubaensis*, possesses immunodepressant properties similar to but more potent than cyclosporine. It inhibits both cell-mediated and humoral immune responses. Like
- 20 cyclosporine, tacrolimus demonstrates considerable interindividual variation in its pharmacokinetic profile. Most clinical studies with tacrolimus have neither been published in their entirety nor subjected to extensive peer review; there is also a paucity of published randomized investigations of tacrolimus vs. cyclosporine, particularly in renal transplantation. Despite these drawbacks,
- 25 tacrolimus has shown notable efficacy as a rescue or primary immunosuppressant therapy when combined with corticosteroids. The potential for reductional withdrawal of corticosteroid therapy with tacrolimus appears to be a distinct advantage compared with the cyclosporine. This benefit may be enhanced by reduced incidence of infectious complications,
- 30 hypertension and hypercholesterolemia reported by some investigators. In other respects, the tolerability profile of tacrolimus appears to be broadly similar to that of cyclosporine.

In addition to induction of graft tolerance, T cell anergy can be used to reverse autoimmune diseases. Autoimmune diseases represent a broad category of conditions. A few examples include insulin-dependent diabetes mellitus (IDDM), multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), myasthenia gravis (MG), and psoriasis. These seemingly disparate diseases all share the common feature of inappropriate immune response to specific self-antigens. Finck et al. *supra* have reported that CTLA4Ig treatment of mice blocked auto-antibody production in a mice model of SLE. In fact, this effect was observed even when the CTLA4Ig treatment was initiated during the advanced stages of the disease, suggesting that the autoimmune response was a reversible process.

Chappel, WO 94/11011 describes methods to treat autoimmune diseases by inducing tolerance to cells, tissues and organs. The application states that—

- 15 "Cells genetically engineered with DNA encoding a plurality of antigens of a cell, tissue, or organ to which tolerance is to be induced. The cells are free of co-stimulatory antigens, such as B7 antigen. Such cells induce T-cell anergy against the proteins encoded by the DNA, and may be administered to a patient in order to prevent the onset of or to treat an autoimmune disease, or to induce tolerance to a tissue or organ prior to transplantation."
- 20 Allergic reactions represent an immediate hypersensitivity response to environmental antigens, typically mediated by IgE antibodies. The ability to induce antigen-specific tolerance provides a powerful avenue to alleviate allergies by exposure to the antigen in conjunction with down-regulation of B7-1, B7-2, B7-3 or CD40.
- 25 The specific roles of B7-1, B7-2 and B7-3 in T cell activation remains to be determined. Some studies suggest that their functions are essentially redundant (Hathcock et al 1994 *J Exp. Med.* 180, 631), or that the differences observed in the kinetics of expression might simply indicate that B7-2 is important in the initiation of the co-stimulatory signal, while B7-1 plays a role in the amplification of that signal. Other studies point to more specific functions.
- 30 For example, Kuchroo et al., 1995 *Cell* 80, 707, have reported that blocking B7-1 expression may favor a Th2 response, while blocking B7-2 expression favors a Th1 response. These two helper T cell subpopulations play distinct roles in the immune response and inflammatory disease. Th1 cells are

strongly correlated with auto-immune disease. Allergic responses are typically triggered by Th2 response. Therefore, the decision to target B7-1, B7-2, CD40 or a combination of the above will depend to the particular disease application.

5

Summary of the Invention

Applicant notes that the inhibition of collagenase and stromelysin production in the synovial membrane of joints can be accomplished using ribozymes and antisense molecules. Ribozyme treatment can be a partner to
10 current treatments which primarily target immune cells reacting to pre-existing tissue damage. Early ribozyme or antisense treatment which reduces the collagenase or stromelysin-induced damage can be followed by treatment with the anti-inflammatories or retinoids, if necessary. In this manner, expression of the proteinases can be controlled at both transcriptional and
15 translational levels. Ribozyme or antisense treatment can be given to patients expressing radiological signs of osteoarthritis prior to the expression of clinical symptoms. Ribozyme or antisense treatment can impact the expression of stromelysin without introducing the non-specific effects upon gene expression which accompany treatment with the retinoids and dexamethasone. The
20 ability of stromelysin to activate procollagenase indicates that a ribozyme or antisense molecule which reduces stromelysin expression can also be used in the treatment of both osteoarthritis (which is primarily a stromelysin-associated pathology) and rheumatoid arthritis (which is primarily related to enhanced collagenase activity).

25 While a number of cytokines and growth factors induce metalloproteinase activities during wound healing and tissue injury of a pre-osteoarthritic condition, these molecules are not preferred targets for therapeutic intervention. Primary emphasis is placed upon inhibiting the molecules which are responsible for the disruption of the extracellular matrix,
30 because most people will be presenting radiologic or clinical symptoms prior to treatment. The most versatile of the metalloproteinases (the molecule which can do the most structural damage to the extracellular matrix, if not regulated)

is stromelysin. Additionally, this molecule can activate procollagenase, which in turn causes further damage to the collagen backbone of the extracellular matrix. Under normal conditions, the conversion of prostromelysin to active stromelysin is regulated by the presence of inhibitors called TIMPs (tissue inhibitors of MMP). Because the level of TIMP in synovial cells exceeds the level of prostromelysin and stromelysin activity is generally absent from the synovial fluid associated with non-arthritic tissues, the toxic effects of inhibiting stromelysin activity in non-target cells should be negligible.

Thus, the invention features use of specific ribozyme molecules to treat or prevent arthritis, particularly osteoarthritis, by inhibiting the synthesis of the prostromelysin molecule in synovial cells, or by inhibition of other matrix metalloproteinases discussed above. Cleavage of targeted mRNAs (stromelysin mRNAs, including stromelysin 1, 2, and 3, and collagenase) expressed in macrophages, neutrophils and synovial cells represses the synthesis of the zymogen form of stromelysin, prostromelysin.

Ribozymes are RNA molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence specific manner. It is said that such enzymatic RNA molecules can be targeted to virtually any RNA transcript and efficient cleavage has been achieved *in vitro*. Kim et al., 84 Proc. Nat. Acad. of Sci. USA 8788, 1987; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acid Research 1371, 1989.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds *trans* (and thus can cleave other RNA molecules) under physiological conditions. Table I summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct

synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

By "enzymatic RNA molecule" it is meant an RNA molecule which has
5 complementarity in a substrate binding region to a specified mRNA target, and also has an enzymatic activity which is active to specifically cleave that mRNA. That is, the enzymatic RNA molecule is able to intermolecularly cleave mRNA and thereby inactivate a target mRNA molecule. This complementarity
10 functions to allow sufficient hybridization of the enzymatic RNA molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. For *in vivo* treatment, complementarity between 30 and 45 bases is preferred; although lower numbers are also useful.

By "complementary" is meant a nucleotide sequence that can form
15 hydrogen bond(s) with other nucleotide sequence by either traditional Watson-Crick or other non-traditional types (for example Hoogsteen type) of base-paired interactions.

The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule
20 simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly
25 specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not
30 prevent their action (Woolf, T. M., et al., 1992, Proc. Natl. Acad. Sci. USA, 89, 7305-7309). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or *Neurospora* VS RNA. Examples
5 of such hammerhead motifs are described by Rossi *et al.*, 1992, Aids Research and Human Retroviruses 8, 183, of hairpin motifs by Hampel *et al.*, EPA 0360257, Hampel and Tritz, 1989 Biochemistry 28, 4929, and Hampel *et al.*, 1990 Nucleic Acids Res. 18, 299, and an example of the hepatitis delta virus motif is described by Perrotta and Been, 1992 Biochemistry 31, 16; of the
10 RNaseP motif by Guerrier-Takada *et al.*, 1983 Cell 35, 849, *Neurospora* VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990 Cell 61, 685-696; Saville and Collins, 1991 Proc. Natl. Acad. Sci. USA 88, 8826-8830; Collins and Olive, 1993 Biochemistry 32, 2795-2799) and of the Group I intron by Cech *et al.*, U.S. Patent 4,987,071. These specific motifs are not
15 limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

20 The invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target stromelysin encoding mRNAs such that specific treatment of a disease or condition can be provided with
25 either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA or RNA vectors that are delivered to specific cells.

Synthesis of nucleic acids greater than 100 nucleotides in length is
30 difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small enzymatic nucleic acid motifs (*e.g.*, of the hammerhead or the hairpin structure) are used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. However,

these catalytic RNA molecules can also be expressed within cells from eukaryotic promoters (e.g., Scanlon et al., 1991, Proc. Natl. Acad. Sci. USA, 88, 10591-5; Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Dropulic et al., 1992 J. Virol., 66, 1432-41; Weerasinghe et al., 1991 J. Virol., 65, 5531-4; Ojwang et al., 1992 Proc. Natl. Acad. Sci. USA, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Sarver et al., 1990 Science 247, 1222-1225; Thompson et al., 1995 Nucleic Acids Res. 23, 2259). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO 93/23569, and Sullivan et al., PCT WO 94/02595; Ohkawa et al., 1992 Nucleic Acids Symp. Ser., 27, 15-6; Taira et al., 1991, Nucleic Acids Res., 19, 5125-30; Ventura et al., 1993 Nucleic Acids Res., 21, 3249-55; Chowrira et al., 1994 J. Biol. Chem. 269, 25856) .

Ribozyme therapy, due to its exquisite specificity, is particularly well-suited to target mRNA encoding factors that contribute to disease pathology. Thus, ribozymes that cleave stromelysin mRNAs may represent novel therapeutics for the treatment of asthma.

Thus, in a first aspect, the invention features ribozymes that inhibit stromelysin production. These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the cleavage of RNA. The RNA molecules are preferably ribozymes of the hammerhead or hairpin motif. Upon binding, the ribozymes cleave the target stromelysin encoding mRNAs, preventing translation and stromelysin protein accumulation. In the absence of the expression of the target gene, a therapeutic effect may be observed.

By "inhibit" is meant that the activity or level of stromelysin encoding mRNAs and protein is reduced below that observed in the absence of the ribozyme, and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the level of stromelysin activity in a cell or tissue. By "related" is meant that the inhibition of stromelysin mRNAs and thus reduction in the level of stromelysin activity will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, aerosol inhalation, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables AII, AIII, AIV, AVI, AVIII and AIX. Examples of such ribozymes are shown in Tables AV, AVII, AVIII and AIX. Examples of such ribozymes consist essentially of sequences defined in these Tables.

By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

In a related aspect the invention features ribozymes that cleave target molecules and inhibit stromelysin activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

This class of chemicals exhibits a high degree of specificity for cleavage of the intended target mRNA. Consequently, the ribozyme agent will only
5 affect cells expressing that particular gene, and will not be toxic to normal tissues.

The invention can be used to treat or prevent (prophylactically) osteoarthritis or other pathological conditions which are mediated by metalloproteinase activation. The preferred administration protocol is *in vivo*
10 administration to reduce the level of stromelysin activity.

Thus, the invention features an enzymatic RNA molecule (or ribozyme) which cleaves mRNA associated with development or maintenance of an arthritic condition, e.g., mRNA encoding stromelysin, and in particular, those
15 mRNA targets disclosed in the accompanying tables, which include both hammerhead and hairpin target sites. In each case the site is flanked by regions to which appropriate substrate binding arms can be synthesized and an appropriate hammerhead or hairpin motif can be added to provide enzymatic activity, by methods described herein and known in the art. For example, referring to Figure 1, arms I and III are modified to be specific
20 substrate-binding arms, and arm II remains essentially as shown.

Ribozymes that cleave stromelysin mRNAs represent a novel therapeutic approach to arthritic disorders like osteoarthritis. The invention features use of ribozymes to treat osteoarthritis, e.g., by inhibiting the synthesis of prostromelysin molecule in synovial cells or by the inhibition of matrix
25 metalloproteinases. Applicant indicates that ribozymes are able to inhibit the secretion of stromelysin and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave stromelysin encoding mRNAs may be readily designed and are within the
30 invention.

In other related aspects, the invention features a mammalian cell which includes an enzymatic RNA molecule as described above. Preferably, the

mammalian cell is a human cell; and the invention features an expression vector which includes nucleic acid encoding an enzymatic RNA molecule described above, located in the vector, e.g., in a manner which allows expression of that enzymatic RNA molecule within a mammalian cell; or a
5 method for treatment of a disease or condition by administering to a patient an enzymatic RNA molecule as described above.

The invention provides a class of chemical cleaving agents which exhibit a high degree of specificity for the mRNA causative of an arthritic condition. Such enzymatic RNA molecules can be delivered exogenously or
10 endogenously to infected cells. In the preferred hammerhead motif the small size (less than 40 nucleotides, preferably between 32 and 36 nucleotides in length) of the molecule allows the cost of treatment to be reduced.

The enzymatic RNA molecules of this invention can be used to treat arthritic or prearthritic conditions. Such treatment can also be extended to
15 other related genes in nonhuman primates. Affected animals can be treated at the time of arthritic risk detection, or in a prophylactic manner. This timing of treatment will reduce the chance of further arthritic damage.

In another aspect, the invention features novel nucleic acid-based techniques [e.g., enzymatic nucleic acid molecules (ribozymes), antisense
20 nucleic acids, 2-5A antisense chimeras, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups (Cook et al., U.S. Patent 5,359,051)] and methods for their use to induce graft tolerance, to treat autoimmune diseases such as lupus, rheumatoid arthritis, multiple sclerosis and to treatment of allergies.

25 In a preferred embodiment, the invention features use of one or more of the nucleic acid-based techniques to induce graft tolerance by inhibiting the synthesis of B7-1, B7-2, B7-3 and CD40 proteins.

Those in the art will recognize the other potential targets, for e.g., ICAM-1, VCAM-1, β 1 integrin (VLA4) are also suitable for treatment with the nucleic
30 acid-based techniques described in the present invention.

By "inhibit" is meant that the activity of B7-1, B7-2, B7-3 and/or CD40 or level of mRNAs encoded by B7-1, B7-2, B7-3 and/or CD40 is reduced below that observed in the absence of the nucleic acid. In one embodiment, inhibition with ribozymes preferably is below that level observed in the presence of an enzymatically inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

By "equivalent" RNA to B7-1, B7-2, B7-3 and/or CD40 is meant to include those naturally occurring RNA molecules associated with graft rejection in various animals, including human, mice, rats, rabbits, primates and pigs.

By "antisense nucleic acid" is meant a non-enzymatic nucleic acid molecule that binds to another RNA (target RNA) by means of RNA-RNA or RNA-DNA or RNA-PNA (protein nucleic acid; Egholm et al., 1993 *Nature* 365, 566) interactions and alters the activity of the target RNA (for a review see Stein and Cheng, 1993 *Science* 261, 1004).

By "2-5A antisense chimera" is meant, an antisense oligonucleotide containing a 5' phosphorylated 2'-5'-linked adenylate residues. These chimeras bind to target RNA in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease which in turn cleaves the target RNA (Torrence et al., 1993 *Proc. Natl. Acad. Sci. USA* 90, 1300).

By "triplex DNA" is meant an oligonucleotide that can bind to a double-stranded DNA in a sequence-specific manner to form a triple-strand helix. Triple-helix formation has been shown to inhibit transcription of the targeted gene (Duval-Valentin et al., 1992 *Proc. Natl. Acad. Sci. USA* 89, 504).

By "gene" is meant a nucleic acid that encodes an RNA.

Ribozymes that cleave the specified sites in B7-1, B7-2, B7-3 and/or CD40 mRNAs represent a novel therapeutic approach to induce graft tolerance and treat autoimmune diseases, allergies and other inflammatory conditions. Applicant indicates that ribozymes are able to inhibit the activity of B7-1, B7-2, B7-3 and/or CD40 and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave these

sites in B7-1, B7-2, B7-3 and/or CD40 mRNAs may be readily designed and are within the invention.

In a preferred embodiment the invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNAs encoding B7-1, B7-2, B7-3 and/or CD40 proteins such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA/RNA vectors that are delivered to specific cells.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the levels of B7-1, B7-2, B7-3 and/or CD40 activity in a cell or tissue. By "related" is meant that the inhibition of B7-1, B7-2, B7-3 and/or CD40 mRNAs and thus reduction in the level respective protein activity will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Examples of such ribozymes are shown in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Examples of such ribozymes consist essentially of sequences defined in these Tables.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit B7-1, B7-2, B7-3 and/or CD40 activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-

associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

The drawings will first briefly be described.

Drawings

Figure 1 is a diagrammatic representation of the hammerhead ribozyme domain known in the art. Stem II can be ≥ 2 base-pairs long.

Figure 2a is a diagrammatic representation of the hammerhead ribozyme domain known in the art; Figure 2b is a diagrammatic representation of the hammerhead ribozyme as divided by Uhlenbeck (1987, *Nature*, 327, 596-600) into a substrate and enzyme portion; Figure 2c is a similar diagram showing the hammerhead divided by Haseloff and Gerlach (1988, *Nature*, 334, 585-591) into two portions; and Figure 2d is a similar diagram showing the hammerhead divided by Jeffries and Symons (1989, *Nucl. Acids. Res.*, 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the general structure of a hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, n is 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or more bases

(preferably 3 - 20 bases, *i.e.*, *m* is from 1 - 20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (*i.e.*, *r* is ≥ 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4 - 20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each *N* and *N'* independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (*i.e.*, *o* and *p* is each independently from 0 to any number, *e.g.*, 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, *i.e.*, without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H, refers to bases A, U or C. Y refers to pyrimidine bases. "—" refers to a chemical bond.

Figure 4 is a representation of the general structure of the hepatitis delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 is a schematic representation of an RNaseH accessibility assay. Specifically, the left side of Figure 6 is a diagram of complementary DNA oligonucleotides bound to accessible sites on the target RNA. Complementary DNA oligonucleotides are represented by broad lines labeled A, B, and C. Target RNA is represented by the thin, twisted line. The right side of Figure 6 is a schematic of a gel separation of uncut target RNA from a cleaved target RNA. Detection of target RNA is by autoradiography of body-labeled, T7 transcript. The bands common to each lane represent uncleaved target RNA; the bands unique to each lane represent the cleaved products.

Figure 7 shows *in vitro* cleavage of stromelysin mRNA by HH ribozymes.

Figure 8 shows inhibition of stromelysin expression by 21HH ribozyme in HS-27 fibroblast cell line.

Figure 9 shows inhibition of stromelysin expression by 463HH ribozyme
5 in HS-27 fibroblast cell line.

Figure 10 shows inhibition of stromelysin expression by 1049HH ribozyme in HS-27 fibroblast cell line.

Figure 11 shows inhibition of stromelysin expression by 1366HH ribozyme in HS-27 fibroblast cell line.

10 Figure 12 shows inhibition of stromelysin expression by 1410HH ribozyme in HS-27 fibroblast cell line.

Figure 13 shows inhibition of stromelysin expression by 1489HH ribozyme in HS-27 fibroblast cell line.

Figure 14 shows 1049HH ribozyme-mediated reduction in the level of
15 stromelysin mRNA in rabbit knee.

Figure 15 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 16 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

20 Figure 17 shows the effect of phosphorothioate substitutions on the catalytic activity of 1049 2'-C-allyl HH ribozyme. A) diagrammatic representation of 1049 hammerhead ribozyme•substrate complex. 1049 U4-C-allyl P=S ribozyme represents a hammerhead containing ribose residues at five positions. The remaining 31 nucleotide positions contain 2'-hydroxyl
25 group substitutions, wherein 30 nucleotides contain 2'-O-methyl substitutions and one nucleotide (U₄) contains 2'-C-allyl substitution. Additionally, five nucleotides within the ribozyme, at the 5' and 3' termini, contain

phosphorothioate substitutions. B) shows the ability of ribozyme described in Fig. 17A to decrease the level of stromelysin RNA in rabbit knee.

Figure 18 is a diagrammatic representation of chemically modified ribozymes targeted against stromelysin RNA. 1049 2'-amino P=S Ribozyme
5 represents a hammerhead containing ribose residues at five positions. The remaining 31 nucleotide positions contain 2'-hydroxyl group substitutions, wherein 29 nucleotides contain 2'-O-methyl substitutions and two nucleotides (U₄ and U₇) contain 2'-amino substitution. Additionally, the 3' end of this ribozyme contains a 3'-3' linked inverted T and four nucleotides at the 5'
10 termini contain phosphorothioate substitutions. Arrow-head indicates the site of RNA cleavage (site 1049). 1363 2'-Amino P=S, Human and Rabbit 1366 2'-Amino P=S ribozymes are identical to the 1049 2'-amino P=S ribozyme except that they are targeted to sites 1363 and 1366 within stromelysin RNAs.

Figure 19 shows 1049 2'-amino P=S ribozyme-mediated reduction in the
15 level of stromelysin mRNA in rabbit knee.

Figure 20 shows 1363 2'-amino P=S ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 21 shows 1366 2'-amino P=S ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

20 Figures 22a-d are diagrammatic representations of non-limiting examples of base modifications for adenine, guanine, cytosine and uracil, respectively.

Figure 23 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al.*, *Nucleic Acids Res.* 1992,
25 20:3252) showing specific substitutions in the catalytic core and substrate binding arms. Compounds 4, 9, 13, 17, 22 and 23 are described in Fig. 24.

Figure 24 is a diagrammatic representation of various nucleotides that can be substituted in the catalytic core of a hammerhead ribozyme.

Figure 25 is a diagrammatic representation of the synthesis of a
30 ribothymidine phosphoramidite.

Figure 26 is a diagrammatic representation of the synthesis of a 5-methylcytidine phosphoramidite.

Figure 27 is a diagrammatic representation of the synthesis of 5-bromouridine phosphoramidite.

5 Figure 28 is a diagrammatic representation of the synthesis of 6-azauridine phosphoramidite.

Figure 29 is a diagrammatic representation of the synthesis of 2,6-diaminopurine phosphoramidite.

10 Figure 30 is a diagrammatic representation of the synthesis of a 6-methyluridine phosphoramidite.

Figure 31 is a representation of a hammerhead ribozyme targeted to site A (HH-A). Site of 6-methyl U substitution is indicated.

15 Figure 32 shows RNA cleavage reaction catalyzed by HH-A ribozyme containing 6-methyl U-substitution (6-methyl-U4). U4, represents a HH-A ribozyme containing no 6-methyl-U substitution.

Figure 33 is a representation of a hammerhead ribozyme targeted to site B (HH-B). Sites of 6-methyl U substitution are indicated.

20 Figure 34 shows RNA cleavage reaction catalyzed by HH-B ribozyme containing 6-methyl U-substitutions at U4 and U7 positions (6-methyl-U4). U4, represents a HH-B ribozyme containing no 6-methyl-U substitution.

Figure 35 is a representation of a hammerhead ribozyme targeted to site C (HH-C). Sites of 6-methyl U substitution are indicated.

25 Figure 36 shows RNA cleavage reaction catalyzed by HH-C ribozyme containing 6-methyl U-substitutions at U4 and U7 positions (6-methyl-U4). U4, represents a HH-C ribozyme containing no 6-methyl-U substitution.

Figure 37 shows 6-methyl-U-substituted HH-A ribozyme-mediated inhibition of rat smooth muscle cell proliferation.

Figure 38 shows 6-methyl-U-substituted HH-C ribozyme-mediated inhibition of stromelysin protein production in human synovial fibroblast cells.

Figure 39 is a diagrammatic representation of the synthesis of pyridin-2-one nucleoside and pyridin-4-one nucleoside phosphoramidite.

5 Figure 40 is a diagrammatic representation of the synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-phenyl- β -D-ribofuranose phosphoramidite.

10 Figure 41 is a diagrammatic representation of the synthesis of pseudouridine, 2,4,6-trimethoxy benzene nucleoside and 3-methyluridine phosphoramidite.

Figure 42 is a diagrammatic representation of the synthesis of dihydrouridine phosphoramidite.

15 Figure 43 A) is diagrammatic representation of a hammerhead ribozyme targeted to site B. B) shows RNA cleavage reaction catalyzed by hammerhead ribozyme with modified base substitutions at either position 4 or position 7.

20 Figure 44 shows further kinetic characterization of RNA cleavage reactions catalyzed by HH-B ribozyme (A); HH-B with pyridin-4-one substitution at position 7 (B); and HH-B with phenyl substitution at position 7 (C).

Figure 45 is a diagrammatic representation of the synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-Deoxy-1-Naphthyl- β -D-Ribofuranose.

25 Figure 46 is a diagrammatic representation of the synthesis of Synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-Deoxy-1-(*p*-Aminophenyl)- β -D-Ribofuranose.

Figure 47 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al. Nucleic Acids Res.* 1992, 20, 3252) showing specific substitutions.

5 Figure 48 shows the structures of various 2'-alkyl modified nucleotides which exemplify those of this invention. R groups are alkyl groups, Z is a protecting group.

Figure 49 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine.

10 Figure 50 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene uridine.

Figure 51 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene cytidine.

Figure 52 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene adenosine.

15 Figure 53 is a diagrammatic representation of the synthesis of 2'-C-carboxymethylidene uridine, 2'-C-methoxycarboxymethylidene uridine and derivatized amidites thereof. X is CH₃ or alkyl as discussed above, or another substituent.

20 Figure 54 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine phosphoramidites.

25 Figure 55 is a diagrammatic representation of the synthesis of 2'-O-alkylthioalkyl nucleosides or non-nucleosides and their phosphoramidites. R is an alkyl as defined above. B is any naturally occurring or modified base bearing any N-protecting group suitable for standard oligonucleotide synthesis (Usman *et al.*, *supra*; Scaringe *et al.*, *supra*), and/or H (non-nucleotide), as described by the publications discussed above. CE is cyanoethyl, DMT is a standard blocking group. Other abbreviations are standard in the art.

Figure 56 is a diagrammatic representation of a hammerhead ribozyme, targeted to site B (HH-B), containing 2'-O-methylthiomethyl substitutions.

Figure 57 shows RNA cleavage activity catalyzed by 2'-O-methylthiomethyl substituted ribozymes. A plot of percent cleaved as a function of time is shown. The reactions were carried out at 37°C in the presence of 40 nM ribozyme, 1 nM substrate and 10 mM MgCl₂. Control HH-B ribozyme contained the following modifications; 29 positions were modified with 2'-O-methyl, U4 and U7 positions were modified with 2'-amino groups, 5 positions contained 2'-OH groups. These modifications of the control ribozyme have previously been shown not to significantly effect the activity of the ribozyme (Usman et al., 1994 *Nucleic Acids Symposium Series* 31, 163).

Figure 58 is a diagrammatic representation of the synthesis of an abasic deoxyribose or ribose non-nucleotide mimetic phosphoramidite.

Figure 59 is a diagrammatic representation of a hammerhead ribozyme targeted to site B (HH-B). Arrow indicates the cleavage site.

Figure 60 is a diagrammatic representation of HH-B ribozyme containing abasic substitutions (HH-Ba) at various positions. Ribozymes were synthesized as described in the application. "X" shows the positions of abasic substitutions. The abasic substitutions were either made individually or in certain combinations.

Figure 61 shows the *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. All RNA, refers to HHA ribozyme containing no abasic substitution. U4 Abasic, refers to HH-Ba ribozyme with a single abasic (ribose) substitution at position 4. U7 Abasic, refers to HH-Ba ribozyme with a single abasic (ribose) substitution at position 7.

Figure 62 shows *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. Abasic Stem II Loop, refers to HH-Ba ribozyme with four abasic (ribose) substitutions within the loop in stem II.

Figure 63 shows *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. 3'-Inverted Deoxyribose, refers to HH-Ba ribozyme with an inverted deoxyribose (abasic) substitution at its 3' termini.

5 Figure 64 is a diagrammatic representation of a hammerhead ribozyme targeted to site A (HH-A). Target A is involved in the proliferation of mammalian smooth muscle cells. Arrow indicates the site of cleavage. Inactive version of HH-A contains 2 base-substitutions (G5U and A15.1U) that renders the ribozyme catalytically inactive.

10 Figure 65 is a diagrammatic representation of HH-A ribozyme with abasic substitution (HH-Aa) at position 4. X, shows the position of abasic substitution.

Figure 66 shows ribozyme-mediated inhibition of rat aortic smooth muscle cell (RASMC) proliferation. Both HH-A and HH-Aa ribozymes can inhibit the proliferation of RASMC in culture. Catalytically inactive HH-A ribozyme shows inhibition which is significantly lower than active HH-A and
15 HH-Aa ribozymes.

Figure 67 is a schematic representation of a two pot deprotection protocol with ethylamine (EA).

Figure 68 shows a strategy used in synthesizing a hammerhead ribozyme from two halves. X and Y represent reactive moieties that can
20 undergo a chemical reaction to form a covalent bond (represented by the solid curved line).

Figure 69 shows various non-limiting examples of reactive moieties that can be placed in the nascent loop region to form a covalent bond to provide a full-length ribozyme. CH₂ can be any linking chain as described above
25 including groups such as methylenes, ether, ethylene glycol, thioethers, double bonds, aromatic groups and others; each n independently is an integer from 0 to 10 inclusive and may be the same or different; each R independently is a proton or an alkyl, alkenyl and other functional groups or conjugates such
30 as peptides, steroids, hormones, lipids, nucleic acid sequences and others that provides nuclease resistance, improved cell association, improved cellular uptake or interacellular localization.

Figure 70 shows non-limiting examples of covalent bonds that can be formed to provide the full length ribozyme. The morpholino group arises from reductive reaction of a dialdehyde, which arises from oxidative cleavage of a ribose at the 3'-end of one half ribozyme with an amine at the 5'-end of the half ribozyme. The amide bond is produced when an acid at the 3'-end of one half ribozyme is coupled to an amine at the 5'-end of the other half ribozyme.

Figure 71 shows non-limiting examples of three ribozymes that were synthesized from coupling reactions of two halves. All three were targeted to the site A of *c-myb* RNA (HH-A). HH-A1 was formed from the reaction of two thiols to provide the disulfide linked ribozyme. HH-A2 and HH-A3 were formed using the morpholino reaction. HH-A2 contains a five atom spacer linking the terminal amine to the 5'-end of the half ribozyme. HH-A3 contains a six carbon spacer linking the terminal amine to the 5'-end of the half ribozyme.

Figure 72 shows comparative cleavage activity of half ribozymes, containing five and six base pair stem II regions, that are not covalently linked vs a full length ribozyme. Assays were carried out under ribozyme excess conditions.

Figure 73 shows comparative cleavage activity of half ribozymes, containing seven and eight base pair stem II regions, that are not covalently linked vs a full length ribozyme. Assays were carried out under ribozyme excess conditions.

Figure 74 shows comparative cleavage assay of HH-A1, HH-A2 and HH-A3 (see Figure 72) formed from crosslinking reactions vs a full length ribozyme control. Assays were carried out under ribozyme excess conditions.

Figure 75. Schematic representation of RNA polymerase III promoter structure. Arrow indicates the transcription start site and the direction of coding region. A, B and C, refer to consensus A, B and C box promoter sequences. I, refers to intermediate cis-acting promoter sequence. PSE, refers to proximal sequence element. DSE, refers to distal sequence element. ATF, refers to activating transcription factor binding element. ?, refers to cis-

acting sequence element that has not been fully characterized. EBER, Epstein-Barr-virus-encoded-RNA. TATA is a box well known in the art.

Figure 76 is a general formula for pol III RNA of this invention.

5 Figure 77 is a diagrammatic representation of a U6-S35 Chimera. The S35 motif and the site of insertion of a desired RNA are indicated. This chimeric RNA transcript is under the control of a U6 small nuclear RNA (snRNA) promoter.

10 Figure 78 is a diagrammatic representation of a U6-S35-ribozyme chimera. The chimera contains a hammerhead ribozyme targeted to site I (HHI).

Figure 79 is a diagrammatic representation of a U6-S35-ribozyme chimera. The chimera contains a hammerhead ribozyme targeted to site II (HHII).

15 Figure 80 shows RNA cleavage reaction catalyzed by a synthetic hammerhead ribozyme (HHI) and by an *in vitro* transcript of U6-S35-HHI hammerhead ribozyme.

Figure 81 shows stability of U6-S35-HHII RNA transcript in 293 mammalian cells as measured by actinomycin D assay.

20 Figure 82 is a diagrammatic representation of an adenovirus VA1 RNA. Various domains within the RNA secondary structure are indicated.

25 Figure 83 A shows a secondary structure model of a VA1-S35 chimeric RNA containing the promoter elements A and B box. The site of insertion of a desired RNA and the S35 motif are indicated. The transcription unit also contains a stable stem (S35-like motif) in the central domain of the VA1 RNA which positions the desired RNA away from the main transcript as an independent domain. 83B shows a VA1-chimera which consists of the terminal 75 nt of a VA1 RNA followed by the HHI ribozyme.

Figure 84 shows a comparison of stability of VA1-chimeric RNA vs VA1-S35-chimeric RNA as measured by actinomycin D assay. VA1-chimera

consists of terminal 75 nt of VA1 RNA followed by HHI ribozyme. VA1-S35 chimera structure and sequence is shown in Figure 83.

Ribozymes

5 Ribozymes in one aspect of this invention block to some extent stromelysin expression and can be used to treat disease or diagnose such disease. Ribozymes are delivered to cells in culture and to cells or tissues in animal models of osteoarthritis (Hembry et al., 1993 Am. J. Pathol. 143, 628). Ribozyme cleavage of stromelysin encoding mRNAs in these systems may prevent inflammatory cell function and alleviate disease symptoms.

10 Other ribozymes of this invention block to some extent B7-1, B7-2, B7-3 and/or CD40 production and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture, to cells or tissues in animal models of transplantation, autoimmune diseases and/or allergies and to human cells or tissues *ex vivo* or *in vivo*. Ribozyme cleavage of B7-1, B7-2
15 and/or CD40 encoded mRNAs in these systems may alleviate disease symptoms.

Target sites

Targets for useful ribozymes can be determined as disclosed in Draper et al supra, Sullivan et al., supra, as well as by Draper et al., WO 95/13380 and
20 Stinchcomb et al WO 95/23225. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. Such ribozymes can also be optimized
25 and delivered as described therein. While specific examples to mouse, rabbit and other animal RNA are provided, those in the art will recognize that the equivalent human RNA targets described can be used as described below. Thus, the same target may be used, but binding arms suitable for targeting human RNA sequences are present in the ribozyme. Such targets may also
30 be selected as described below.

The sequence of human and rabbit stromelysin mRNA were screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables All, AIII, AIV, AVI, AVIII and AIX (All sequences are 5' to 3' in the tables.). While rabbit and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, rabbit targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Similarly, the sequence of human and mouse B7-1, B7-2, B7-3 and/or CD40 mRNAs were screened for optimal ribozyme target sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX (All sequences are 5' to 3' in the tables) The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes may be useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Hammerhead or hairpin ribozymes are designed that could bind and are individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci. USA, 86, 7706-7710) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Referring to Figure 6, mRNA is screened for accessible cleavage sites by the method described generally in Draper WO 93/23569. Briefly, DNA oligonucleotides representing potential hammerhead or hairpin ribozyme cleavage sites are synthesized. A polymerase chain reaction is used to

generate a substrate for T7 RNA polymerase transcription from human or rabbit stromelysin cDNA clones. Labeled RNA transcripts are synthesized *in vitro* from the two templates. The oligonucleotides and the labeled transcripts are annealed, RNaseH is added and the mixtures are incubated for the designated times at 37°C. Reactions are stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved is determined by autoradiographic quantitation using a PhosphorImaging system. From these data, hammerhead ribozyme sites are chosen as the most accessible.

10 Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman et al., 1987 J. Am. Chem. Soc., 109, 7845-7854 and in Scaringe et al., 1990 Nucleic Acids Res., 18, 5433-5441; 15 Wincott *et al.*, 1995 Nucleic Acids Res., 23, 2677, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U 20 for G5 and a U for A14 (numbering from Hertel et al., 1992 Nucleic Acids Res., 20, 3252) . Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 25 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992 TIBS 17, 34 and Beigelman *et al.*, 1995 J. Biol. Chem., 270, 25702). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Stinchcomb et al, supra) and are resuspended in water.

30 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables AV, AVII, AVIII and AIX and in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the

binding arms) is altered to affect activity. For example, stem loop II sequence of hammerhead ribozymes listed in Tables AV and AVII (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. Similarly, stem-loop AIV sequence of hairpin ribozymes listed in Tables AVI and AVII (5'-CACGUUGUG-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables AV, AVII, AVIII and AIX may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described by Stinchcomb *et al.*, supra. The details will not be repeated here, but include altering the length of the ribozyme binding arms (stems I and III, see Figure 2c), or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Stinchcomb *et al.*, supra, Sproat, European Patent Application 92110298.4 and U.S. Patent 5,334,711; Jennings *et al.*, WO 94/13688 and Beigelman *et al.*, supra which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules). Modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan, *et al.*, supra, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some

indications, ribozymes may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination is locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but
5 are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan et al., supra and Draper et al., supra which have been incorporated by reference herein.

10 In another preferred embodiment, the ribozyme is administered to the site of B7-1, B7-2, B7-3 and/or CD40 expression (APC) in an appropriate liposomal vesicle. APCs isolated from donor (for example) are treated with the ribozyme preparation (or other nucleic acid therapeutics) *ex vivo* and the treated cells are infused into recipient. Alternatively, cells, tissues or organs
15 are directly treated with nucleic acids of the present invention prior to transplantation into a recipient.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a
20 promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase
25 promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. U S A, 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have
30 demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. U S A, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. U S A, 90, 6340-4; L'Huillier et al., 1992 EMBO J. 11, 4411-8; Lisiewicz et

al., 1993 Proc. Natl. Acad. Sci. U. S. A., 90, 8000-4; Thompson *et al.*, *supra*). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated vectors), or viral RNA vectors (such as retroviral or alphavirus vectors).

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves stromelysin RNA is inserted into a plasmid DNA vector or an adenovirus DNA virus or adeno-associated virus (AAV) vector. Both viral vectors have been used to transfer genes to the lung and both vectors lead to transient gene expression (Zabner *et al.*, 1993 Cell 75, 207; Carter, 1992 Curr. Opin. Biotech. 3, 533). The adenovirus vector is delivered as recombinant adenoviral particles. The DNA may be delivered alone or complexed with vehicles (as described for RNA above). The recombinant adenovirus or AAV particles are locally administered to the site of treatment, *e.g.*, through incubation or inhalation *in vivo* or by direct application to cells or tissues *ex vivo*.

Specifically useful modifications, optimization and synthetic methods will now be described.

20 Base Modifications

The following discussion of relevant art is dependent on the diagram shown in Figure 1, in which the numbering of various nucleotides in a hammerhead ribozyme is provided.

Odoi *et al.*, *FEBS* 1990, 267:150, state that substitution of guanosine (G) at position 5 of a hammerhead ribozyme for inosine greatly reduces catalytic activity, suggesting "the importance of the 2-amino group of this guanosine for catalytic activity."

Fu and McLaughlin, *Proc. Natl. Acad. Sci. (USA)* 1992, 89:3985, state that deletion of the 2-amino group of the guanosine at position 5 of a hammerhead ribozyme, or deletion of either of the 2'-hydroxyl groups at

position 5 or 8, resulted in ribozymes having a decrease in cleavage efficiency.

5 Fu and McLaughlin, *Biochemistry* 1992, 31:10941, state that substitution of 7-deazaadenosine for adenosine residues in a hammerhead ribozyme can cause reduction in cleavage efficiency. They state that the "results suggest that the N⁷-nitrogen of the adenosine (A) at position 6 in the hammerhead ribozyme/substrate complex is critical for efficient cleavage activity." They go on to indicate that there are five critical functional groups located within the tetrameric sequence GAUG in the hammerhead ribozyme.

10 Slim and Gait, 1992, *BBRC* 183, 605, state that the substitution of guanosine at position 12, in the core of a hammerhead ribozyme, with inosine inactivates the ribozyme.

15 Tuschl *et al.*, 1993 *Biochemistry* 32, 11658, state that substitution of guanosine residues at positions 5, 8 and 12, in the catalytic core of a hammerhead, with inosine, 2-aminopurine, xanthosine, isoguanosine or deoxyguanosine cause significant reduction in the catalytic efficiency of a hammerhead ribozyme.

20 Fu *et al.*, 1993 *Biochemistry* 32, 10629, state that deletion of guanine N⁷, guanine N² or the adenine N⁶-nitrogen within the core of a hammerhead ribozyme causes significant reduction in the catalytic efficiency of a hammerhead ribozyme.

25 Grasby *et al.*, 1993 *Nucleic Acids Res.* 21, 4444, state that substitution of guanosine at positions 5, 8 and 12 positions within the core of a hammerhead ribozyme with O⁶-methylguanosine results in an approximately 75-fold reduction in k_{cat}.

Seela *et al.*, 1993 *Helvetica Chimica Acta* 76, 1809, state that substitution of adenine at positions 13, 14 and 15, within the core of a hammerhead ribozyme, with 7-deazaadenosine does not significantly decrease the catalytic efficiency of a hammerhead ribozyme.

Adams *et al.*, 1994 *Tetrahedron Letters* 35, 765, state that substitution of uracil at position 17 within the hammerhead ribozyme•substrate complex with 4-thiouridine results in a reduction in the catalytic efficiency of the ribozyme by 50 percent.

5 Ng *et al.*, 1994 *Biochemistry* 33, 12119, state that substitution of adenine at positions 6, 9 and 13 within the catalytic core of a hammerhead ribozyme with isoguanosine, significantly decreases the catalytic activity of the ribozyme.

Jennings *et al.*, U.S. Patent 5,298,612, indicate that nucleotides within a
10 "minizyme" can be modified. They state-

"Nucleotides comprise a base, sugar and a
monophosphate group. Accordingly, nucleotide
derivatives or modifications may be made at the
level of the base, sugar or monophosphate
15 groupings..... Bases may be substituted with various
groups, such as halogen, hydroxy, amine, alkyl,
azido, nitro, phenyl and the like."

WO93/23569, WO95/06731, WO95/04818, and WO95/133178 describe
20 various modifications that can be introduced into ribozyme structures.

This invention relates to production of enzymatic RNA molecules or
ribozymes having enhanced or reduced binding affinity and enhanced
enzymatic activity for their target nucleic acid substrate by inclusion of one or
more modified nucleotides in the substrate binding portion of a ribozyme such
25 as a hammerhead, hairpin, VS ribozyme or hepatitis delta virus derived
ribozyme. Applicant has recognized that only small changes in the extent of
base-pairing or hydrogen bonding between the ribozyme and substrate can
have significant effect on the enzymatic activity of the ribozyme on that
substrate. Thus, applicant has recognized that a subtle alteration in the extent
30 of hydrogen bonding along a substrate binding arm of a ribozyme can be used
to improve the ribozyme activity compared to an unaltered ribozyme
containing no such altered nucleotide. Thus, for example, a guanosine base
may be replaced with an inosine to produce a weaker interaction between a
ribozyme and its substrate, or a uracil may be replaced with a bromouracil
35 (BrU) to increase the hydrogen bonding interaction with an adenosine. Other

examples of alterations of the four standard ribonucleotide bases are shown in Figures 22a-d with weaker or stronger hydrogen bonding abilities shown in each figure.

In addition, applicant has determined that base modification within some catalytic core nucleotides maintains or enhances enzymatic activity compared to an unmodified molecule. Such nucleotides are noted in Figure 23. Specifically, referring to Figure 23, the preferred sequence of a hammerhead ribozyme in a 5' to 3' direction of the catalytic core is CUG ANG A G•C GAA A, wherein N can be any base or may lack a base (abasic); G•C is a base-pair. The nature of the base-paired stem II (Figures 1, 2 and 23) and the recognition arms of stems I and III are variable. In this invention, the use of base-modified nucleotides in those regions that maintain or enhance the catalytic activity and/or the nuclease resistance of the hammerhead ribozyme are described. (Bases which can be modified include those shown in capital letters).

Examples of base-substitutions useful in this invention are shown in Figure 22, 24-30, 39-43, 45-46. In preferred embodiments cytidine residues are substituted with 5-alkylcytidines (e.g., 5-methylcytidine, Figure 24, R=CH₃, 9), and uridine residues with 5-alkyluridines (e.g., ribothymidine (Figure 24, R=CH₃, 4) or 5-halouridine (e.g., 5-bromouridine, Figure 24, X=Br, 13) or 6-azapyrimidines (Figure 24, 17) or 6-alkyluridine (Figure 30). Guanosine or adenosine residues may be replaced by diaminopurine residues (Figure 24, 22) in either the core or stems. In those bases where none of the functional groups are important in the complexing of magnesium or other functions of a ribozyme, they are optionally replaced with a purine ribonucleoside (Figure 24, 23), which significantly reduces the complexity of chemical synthesis of ribozymes, as no base-protecting group is required during chemical incorporation of the purine nucleus. Furthermore, as discussed above, base-modified nucleotides may be used to enhance the specificity or strength of binding of the recognition arms with similar modifications. Base-modified nucleotides, in general, may also be used to enhance the nuclease resistance of the catalytic nucleic acids in which they are incorporated. These modifications within the hammerhead ribozyme motif are meant to be non-limiting example. Those skilled in the art will recognize that other ribozyme

motifs with similar modifications can be readily synthesized and are within the scope of this invention.

Substitutions of sugar moieties as described in the art cited above, may also be made to enhance catalytic activity and/or nuclease stability.

5 The invention provides ribozymes having increased enzymatic activity in vitro and in vivo as can be measured by standard kinetic assays. Thus, the kinetic features of the ribozyme are enhanced by selection of appropriate modified bases in the substrate binding arms. Applicant recognizes that while strong binding to a substrate by a ribozyme enhances specificity, it may also
10 prevent separation of the ribozyme from the cleaved substrate. Thus, applicant provides means by which optimization of the base pairing can be achieved. Specifically, the invention features ribozymes with modified bases with enzymatic activity at least 1.5 fold (preferably 2 or 3 fold) or greater than the unmodified corresponding ribozyme. The invention also features a
15 method for optimizing the kinetic activity of a ribozyme by introduction of modified bases into a ribozyme and screening for those with higher enzymatic activity. Such selection may be in vitro or in vivo. By enhanced activity is meant to include activity measured in vivo where the activity is a reflection of both catalytic activity and ribozyme stability. In this invention, the product of
20 these properties is increased or not significantly (less than 10 fold) decreased in vivo compared to an all RNA ribozyme.

By "enzymatic portion" is meant that part of the ribozyme essential for cleavage of an RNA substrate.

25 By "substrate binding arm" is meant that portion of a ribozyme which is complementary to (*i.e.*, able to base-pair with) a portion of its substrate. Generally, such complementarity is 100%, but can be less if desired. For example, as few as 10 bases out of 14 may be base-paired. Such arms are shown generally in Figures 1-3 as discussed below. That is, these arms contain sequences within a ribozyme which are intended to bring ribozyme
30 and target RNA together through complementary base-pairing interactions; *e.g.*, ribozyme sequences within stems I and III of a standard hammerhead ribozyme make up the substrate-binding domain (see Figure 1).

By "unmodified nucleotide base" is meant one of the bases adenine, cytosine, guanosine, uracil joined to the 1' carbon of β -D-ribo-furanose. The sugar also has a phosphate bound to the 5' carbon. These nucleotides are bound by a phosphodiester between the 3' carbon of one nucleotide and the 5' carbon of the next nucleotide to form RNA.

By "modified nucleotide base" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base which has an effect on the ability of that base to hydrogen bond with its normal complementary base, either by increasing the strength of the hydrogen bonding or by decreasing it (e.g., as exemplified above for inosine and bromouracil). Other examples of modified bases include those shown in Figures 22a-d and other modifications well known in the art, including heterocyclic derivatives and the like.

In preferred embodiments the modified ribozyme is a hammerhead, hairpin VS ribozyme or hepatitis delta virus derived ribozyme, and the hammerhead ribozyme includes between 32 and 40 nucleotide bases. The selection of modified bases is most preferably chosen to enhance the enzymatic activity (as observed in standard kinetic assays designed to measure the kinetics of cleavage) of the selected ribozyme, i.e., to enhance the rate or extent of cleavage of a substrate by the ribozyme, compared to a ribozyme having an identical nucleotide base sequence without any modified base.

By "kinetic assays" or "kinetics of cleavage" is meant an experiment in which the rate of cleavage of target RNA is determined. Often a series of assays are performed in which the concentrations of either ribozyme or substrate are varied from one assay to the next in order to determine the influence of that parameter on the rate of cleavage.

By "rate of cleavage" is meant a measure of the amount of target RNA cleaved as a function of time.

Enzymatic nucleic acid having a hammerhead configuration and modified bases which maintain or enhance enzymatic activity are provided. Such nucleic acid is also generally more resistant to nucleases than

unmodified nucleic acid. By "modified bases" in this aspect is meant those shown in Figure 22 A-D, and 24, 30, and 42B or their equivalents; such bases may be used within the catalytic core of the enzyme as well as in the substrate-binding regions. In particular, the invention features modified ribozymes having a base substitution selected from pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyluracil, dihydrouracil, naphthyl, 6-methyl-uracil and aminophenyl. As noted above, substitution in the core may decrease in vitro activity but enhances stability. Thus, in vivo the activity may not be significantly lowered. As exemplified herein such ribozymes are useful in vivo even if active over all is reduced 10 fold. Such ribozymes herein are said to "maintain" the enzymatic activity on all RNA ribozyme.

Small scale synthesis were conducted on a 394 Applied Biosystems, Inc. synthesizer using a modified 2.5 μ mol scale protocol with a 5 min coupling step for alkylsilyl protected nucleotides and 2.5 min coupling step for 2'-O-methylated nucleotides. Table CII outlines the amounts, and the contact times, of the reagents used in the synthesis cycle. A 6.5-fold excess (163 μ L of 0.1 M = 16.3 μ mol) of phosphoramidite and a 24-fold excess of S-ethyl tetrazole (238 μ L of 0.25 M = 59.5 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, were 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer: detritylation solution was 2% TCA in methylene chloride (ABI); capping was performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution was 16.9 mM I_2 , 49 mM pyridine, 9% water in THF (Millipore). B & J Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from American International Chemical, Inc.

Deprotection of the RNA was performed as follows. The polymer-bound oligoribonucleotide, trityl-off, was transferred from the synthesis column to a 4mL glass screw top vial and suspended in a solution of methylamine (MA) at 65 °C for 10 min. After cooling to -20 °C, the supernatant was removed from the polymer support. The support was washed three times with 1.0 mL of

EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder.

5 The base-deprotected oligoribonucleotide was resuspended in anhydrous TEA•HF/NMP solution (250 µL of a solution of 1.5mL *N*-methylpyrrolidinone, 750 µL TEA and 1.0 mL TEA•3HF to provide a 1.4M HF concentration) and heated to 65°C for 1.5 h. The resulting, fully deprotected, oligomer was quenched with 50 mM TEAB (9 mL) prior to anion exchange desalting.

10 For anion exchange desalting of the deprotected oligomer, the TEAB solution was loaded onto a Qiagen 500® anion exchange cartridge (Qiagen Inc.) that was prewashed with 50 mM TEAB (10 mL). After washing the loaded cartridge with 50 mM TEAB (10 mL), the RNA was eluted with 2 M TEAB (10 mL) and dried down to a white powder.

15 Inactive hammerhead ribozymes were synthesized by substituting a U for G₅ and a U for A₁₄ (numbering from (Hertel, K. J., *et al.*, 1992, *Nucleic Acids Res.*, 20, 3252)).

The average stepwise coupling yields were >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684).

20 Hairpin ribozymes are synthesized either as one part or in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840).

Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Stinchcomb *et al.*, International PCT Publication No. WO 95/23225, and are resuspended in water.

25 Various modifications to ribozyme structure can be made to enhance the utility of ribozymes. Such modifications will enhance shelf-life, half-life in vitro, stability, and ease of introduction of such ribozymes to the target site, e.g., to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Examples of such ribozymes are provided in Usman et al., WO 95/13378 and below.

2'-deoxy-2'-nucleotides

Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Stinchcomb *et al.*, *supra*, Sproat, European Patent Application 92110298.4 and U.S. Patent 5,334,711; Jennings *et al.*, WO 94/13688 and Beigelman *et al.*, *supra* which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. Usman *et al.* also describe various required ribonucleotides in a ribozyme, and methods by which such nucleotides can be defined. De Mesmaeker *et al.* Syn. Lett. 1993, 677-680 (not admitted to be prior art to the present invention) describes the synthesis of certain 2'-C-alkyl uridine and thymidine derivatives. They conclude that "...their use in an antisense approach seems to be very limited."

This invention relates to the use of 2'-deoxy-2'-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 2'-deoxy-2'-alkylnucleotide-containing enzymatic nucleic acids are catalytic nucleic acid molecules that contain 2'-deoxy-2'-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 2'-deoxy-2'-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Contrary to the findings of De Mesmaeker *et al.* applicant has found that such

nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 2'-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 2'-deoxy-2'-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures 48-54), and to methods for their synthesis.

Thus, the invention features 2'-deoxy-2'-alkylnucleotides, that is a nucleotide base having at the 2'-position on the sugar molecule an alkyl moiety and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

Examples of various alkyl groups useful in this invention are shown in Figure 48, where each R group is any alkyl. These examples are not limiting in the invention. Specifically, an "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may

be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino or SH. The term "alkyl" does not include alkoxy groups which have an "-O-alkyl" group, where "alkyl" is defined as described above, where the O is adjacent the 2'-position of the sugar molecule.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-deoxy-2'-alkylnucleotides (preferably not a 2'-alkyl- uridine or thymidine); e.g. enzymatic nucleic acids having a 2'-deoxy-2'-alkylnucleotide; and a method for producing an

enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group. In other related aspects, the invention features 2'-deoxy-2'-alkylnucleotide triphosphates.

- 5 These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-alkyl derivatives of this invention provide enhanced stability to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall activity *in vivo*.
10 Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

- In another aspect, the invention features hammerhead motifs having enzymatic activity having ribonucleotides at locations shown in Figure 47 at 5, 6, 8, 12, and 15.1, and having substituted ribonucleotides at other positions in
15 the core and in the substrate binding arms if desired. (The term "core" refers to positions between bases 3 and 14 in Figure 47, and the binding arms correspond to the bases from the 3'-end to base 15.1, and from the 5'-end to base 2). Applicant has found that use of ribonucleotides at these five locations in the core provide a molecule having sufficient enzymatic activity even when
20 modified nucleotides are present at other sites in the motif. Other such combinations of useful ribonucleotides can be determined as described by Usman *et al. supra*.

2'-O-alkylthioalkyl and 2'-C-alkylthioalkyl containing nucleic acids

- Medina et al., 1988 *Tetrahedron Letters* 29, 3773, describe a method to
25 convert alcohols to methylthiomethyl ethers.

Matteucci et al., 1990 *Tetrahedron Letters*, 31, 2385, report the synthesis of 3'-5'-methylene bond via a methylthiomethyl precursor.

- Veeneman et al., 1990 *Recl. Trav. Chim. Pays-Bas* 109, 449, report the synthesis of 3'-O-methylthiomethyl deoxynucleoside during the synthesis of a
30 dimer containing 3'-5'-methylene bond.

Jones et al., 1993 *J. Org. Chem.* 58, 2983, report the use of 3'-O-methylthiomethyl deoxynucleoside to synthesize a dimer containing a 3'-thioformacetal internucleoside linkages. The paper also describes a method to synthesize phosphoramidites for DNA synthesis.

- 5 Zavgorodny et al., 1991 *Tetrahedron Letters* 32, 7593, describe a method to synthesize a nucleoside containing methylthiomethyl modification.

This invention relates to the incorporation of 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides into nucleic acids, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and
10 also as antisense oligonucleotides.

As the term is used in this application, 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide or non-nucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide or non-nucleotides components replacing one or
15 more bases or regions including, but not limited to, those bases in double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can
20 also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

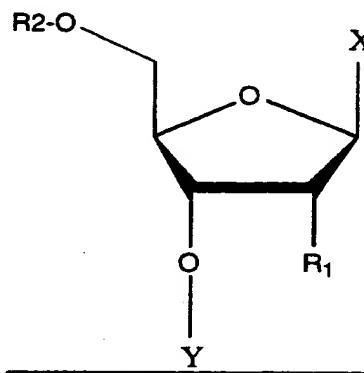
Also within the invention are 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides which may be present in enzymatic nucleic acid or in antisense oligonucleotides or 2-5A antisense chimera. Such
25 nucleotides or non-nucleotides are useful since they enhance the activity of the antisense or enzymatic molecule. The invention also relates to novel intermediates useful in the synthesis of such nucleotides or non-nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

30 Thus, the invention features 2'-O-alkylthioalkyl nucleosides or non-nucleosides, that is a nucleoside or non-nucleosides having at the 2'-position on the sugar molecule a 2'-O-alkylthioalkyl moiety. In a related aspect, the

invention also features 2'-O-alkylthioalkyl nucleotides or non-nucleotides. That is, the invention preferably includes those nucleotides or non-nucleotides having 2' substitutions as noted above useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed
5 above.

The term non-nucleotide refers to any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is
10 abasic in that it does not contain a commonly recognized nucleotide base, such as adenine, guanine, cytosine, uracil or thymine. It may have substitutions for a 2' or 3' H or OH as described in the art. See Eckstein et al. and Usman et al., *supra*.

The term nucleotide refers to the regular nucleotides (A, U, G, T and C)
15 and modified nucleotides such as 6-methyl U, inosine, 5-methyl C and others. Specifically, the term "nucleotide" is used as recognized in the art to include natural bases, and modified bases well known in the art. Such bases are generally located at the 1' position of a sugar moiety. The term "non-nucleotide" as used herein to encompass sugar moieties lacking a base or
20 having other chemical groups in place of a base at the 1' position. Such molecules generally include those having the general formula:



wherein, R1 represents 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl; X represents a base or H; Y represents a phosphorus-containing group; and R2
25 represents H, DMT or a phosphorus-containing group (Figure 55).

Phosphorus-containing group is generally a phosphate, thiophosphate, H-phosphonate, methylphosphonate, phosphoramidite or other modified group known in the art.

5 In another aspect, the invention features 2'-C-alkylthioalkyl nucleosides or non-nucleosides, that is a nucleotide or a non-nucleotide residue having at the 2'-position on the sugar molecule a 2'-C-alkylthioalkyl moiety. In a related aspect, the invention also features 2'-C-alkylthioalkyl nucleotides or non-nucleotides. That is, the invention preferably includes all those 2' modified nucleotides or non-nucleotides useful for making enzymatic nucleic acids or
10 antisense molecules as described above that are not described by the art discussed above.

Specifically, an "alkyl" group is as defined above, except that the term includes 2'-O-alkyl moieties.

15 In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides; *e.g.* enzymatic nucleic acids having a 2'-O-methylthiomethyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule,
20 by forming the enzymatic molecule with at least one nucleotide or a non-nucleotide moiety having at its 2'-position an 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl group.

In other related aspects, the invention features 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide triphosphates. These triphosphates can be
25 used in standard protocols to form useful oligonucleotides of this invention.

The 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl derivatives of this invention provide enhanced activity and stability to the oligonucleotides containing them.

30 In yet another preferred embodiment, the invention features oligonucleotides having one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl abasic (non-nucleotide) moieties. For example, enzymatic

nucleic acids having a 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl abasic moiety; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one position having at its 2'-
5 position an 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl group.

In related embodiments, the invention features enzymatic nucleic acids containing one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl substitutions either in the enzymatic portion, substrate binding portion or both, as long as the catalytic activity of the ribozyme is not significantly decreased.

10 In yet another preferred embodiment, the invention features the use of 2'-O-alkylthioalkyl moieties as protecting groups for 2'-hydroxyl positions of ribofuranose during nucleic acid synthesis.

While this invention is applicable to all oligonucleotides, applicant has found that the modified molecules of this invention are particularly useful for
15 enzymatic RNA molecules. Thus, below is provided examples of such molecules. Those in the art will recognize that equivalent procedures can be used to make other molecules without such enzymatic activity. Specifically, Figure 1 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided.

20 Referring to Figure 1, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG [base paired with] CGAAA. In this invention, the use of 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl substituted nucleotides or non-nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead
25 ribozyme is described. Substitutions of any nucleotide with any of the modified nucleotides or non-nucleotides discussed above are possible. Usman *et al.*, *supra* and Sproat *et al.*, *supra* as well as other publications indicate those bases that can be substituted in noted ribozyme motifs. Those in the art can thus determine those bases that may be substituted as described
30 herein with beneficial retainment of enzymatic activity and stability.

Non-nucleotides

Usman, et al., WO 93/15187 in discussing modified structures in ribozymes states:

5 It should be understood that the linkages between
the building units of the polymeric chain may be
linkages capable of bridging the units together for
either in vitro or in vivo. For example the linkage
may be a phosphorous containing linkage, e.g.,
10 phosphodiester or phosphothioate, or may be a
nitrogen containing linkage, e.g., amide. It should
further be understood that the chimeric polymer
may contain non-nucleotide spacer molecules
along with its other nucleotide or analogue units.
15 Examples of spacer molecules which may be used
are described in Nielsen et al. Science, 254:1497-
1500 (1991).

Jennings et al., WO 94/13688 while discussing hammerhead ribozymes lacking the usual stem II base-paired region state:

20 One or more ribonucleotides and/or
deoxyribonucleotides of the group (X)_m, [stem II]
may be replaced, for example, with a linker
selected from optionally substituted
polyphosphodiester (such as poly(1-phospho-3-
25 propanol)), optionally substituted alkyl, optionally
substituted polyamide, optionally substituted glycol,
and the like. Optional substituents are well known
in the art, and include alkoxy (such as methoxy,
ethoxy and propoxy), straight or branch chain lower
30 alkyl such as C₁ - C₅ alkyl), amine, aminoalkyl
(such as amino C₁ - C₅ alkyl), halogen (such as F,
C1 and Br) and the like. The nature of optional
substituents is not of importance, as long as the
resultant endonuclease is capable of substrate
cleavage.

35 Additionally, suitable linkers may comprise
polycyclic molecules, such as those containing
phenyl or cyclohexyl rings. The linker (L) may be a
polyether such as polyphosphopropanediol,
polyethyleneglycol, a bifunctional polycyclic
40 molecule such as a bifunctional pentalene, indene,
naphthalene, azulene, heptalene, biphenylene,
asymindacene, sym-indacene, acenaphthylene,
fluorene, phenalene, phenanthrene, anthracene,
fluoranthene, acephenathrylene, aceanthrylene,

5 triphenylene, pyrene, chrysene, naphthacene, thianthrene, isobenzofuran, chromene, xanthene, phenoxathiin, indolizine, isoindole, 3-H-indole, indole, 1-H-indazole, 4-H-quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, 4- α H-carbazole, carbazole, B-carboline, phenanthridine, acridine, perimidine, phenanthroline, phenazine, phenolthiazine, phenoxazine, which polycyclic
10 compound may be substituted or modified, or a combination of the polyethers and the polycyclic molecules.

The polycyclic molecule may be substituted of polysubstituted with C₁-C₅ alkyl, alkenyl, hydroxyalkyl, halogen or haloalkyl group or with O-A or CH₂-O-A wherein A is H or has the formula CONR'R" wherein R' and R" are the same or different and are hydrogen or a substituted or unsubstituted C₁-C₆ alkyl, aryl, cycloalkyl, or heterocyclic group; or A has the formula -M-NR'R" wherein R' and R" are the same or different and are hydrogen, or a C₁-C₅ alkyl, alkenyl, hydroxyalkyl, or haloalkyl group wherein the halo atom is fluorine, chlorine, bromine, or iodine atom; and -M- is an organic moiety having 1 to 10 carbon atoms and is a branched or straight chain alkyl, aryl, or cycloalkyl group.
15
20
25

In one embodiment, the linker is tetraphosphopropanediol or pentaphosphopropanediol. In the case of polycyclic molecules there will be preferably 18 or more atoms bridging the nucleic acids. More preferably their will be from 30 to 50 atoms bridging, see for Example 5. In another embodiment the linker is a bifunctional carbazole or bifunctional carbazole linked to one or more polyphosphoropropanediol.
30
35

Such compounds may also comprise suitable functional groups to allow coupling through reactive groups on nucleotides."
40

This invention concerns the use of non-nucleotide molecules as spacer elements at the base of double-stranded nucleic acid (e.g., RNA or DNA) stems (duplex stems) or more preferably, in the single-stranded regions, catalytic core, loops, or recognition arms of enzymatic nucleic acids. Duplex
45

stems are ubiquitous structural elements in enzymatic RNA molecules. To facilitate the synthesis of such stems, which are usually connected via single-stranded nucleotide chains, a base or base-pair mimetic may be used to reduce the nucleotide requirement in the synthesis of such molecules, and to confer nuclease resistance (since they are non-nucleic acid components). This also applies to both the catalytic core and recognition arms of a ribozyme. In particular abasic nucleotides (i.e., moieties lacking a nucleotide base, but having the sugar and phosphate portions) can be used to provide stability within a core of a ribozyme, e.g., at U4 or N7 of a hammerhead structure shown in Figure 1.

Thus, the invention features an enzymatic nucleic acid molecule having one or more non-nucleotide moieties, and having enzymatic activity to cleave an RNA or DNA molecule.

Examples of such non-nucleotide mimetics are shown in Figure 58 and their incorporation into hammerhead ribozymes is shown in Figure 60. These non-nucleotide linkers may be either polyether, polyamine, polyamide, or polyhydrocarbon compounds. Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* **1990**, *18*:6353 and *Nucleic Acids Res.* **1987**, *15*:3113; Cload and Schepartz, *J. Am. Chem. Soc.* **1991**, *113*:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* **1991**, *113*:5109; Ma et al., *Nucleic Acids Res.* **1993**, *21*:2585 and *Biochemistry* **1993**, *32*:1751; Durand et al., *Nucleic Acids Res.* **1990**, *18*:6353; McCurdy et al., *Nucleosides & Nucleotides* **1991**, *10*:287; Jäschke et al., *Tetrahedron Lett.* **1993**, *34*:301; Ono et al., *Biochemistry* **1991**, *30*:9914; Arnold et al., International Publication No. WO 89/02439 entitled "Non-nucleotide Linking Reagents for Nucleotide Probes"; and Ferentz and Verdine, *J. Am. Chem. Soc.* **1991**, *113*:4000, all hereby incorporated by reference herein.

In preferred embodiments, the enzymatic nucleic acid includes one or more stretches of RNA, which provide the enzymatic activity of the molecule; linked to the non-nucleotide moiety.

In preferred embodiments, the enzymatic nucleic acid includes one or more stretches of RNA, which provide the enzymatic activity of the molecule,

linked to the non-nucleotide moiety. The necessary ribonucleotide components are known in the art, see, e.g., Usman, supra and Usman et al., Nucl. Acid. Symp. Genes 31:163, 1994.

As the term is used in this application, non-nucleotide-containing enzymatic nucleic acid means a nucleic acid molecule that contains at least one non-nucleotide component which replaces a portion of a ribozyme, *e.g.*, but not limited to, a double-stranded stem, a single-stranded "catalytic core" sequence, a single-stranded loop or a single-stranded recognition sequence. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such molecules can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript. Such molecules also include nucleic acid molecules having a 3' or 5' non-nucleotide, useful as a capping group to prevent exonuclease digestion.

Non-nucleotide mimetics useful in this invention are generally described above and in Usman et al. WO 95/06731. Those in the art will recognize that these mimetics can be incorporated into an enzymatic molecule by standard techniques at any desired location. Suitable choices can be made by standard experiments to determine the best location, *e.g.*, by synthesis of the molecule and testing of its enzymatic activity. The optimum molecule will contain the known ribonucleotides needed for enzymatic activity, and will have non-nucleotides which change the structure of the molecule in the least way possible. What is desired is that several nucleotides can be substituted by one non-nucleotide to save synthetic steps in enzymatic molecule synthesis and to provide enhanced stability of the molecule compared to RNA or even DNA.

Synthesis

This invention relates to the synthesis, deprotection, and purification of enzymatic RNA or modified enzymatic RNA molecules in milligram to kilogram quantities with high biological activity. Such syntheses are generally detailed in Stinchcomb et al., WO 95/23225.

This invention relates to the synthesis, deprotection, and purification of enzymatic RNA or modified enzymatic RNA molecules in milligram to kilogram quantities with high biological activity.

Generally, RNA is synthesized and purified by methodologies based on:
5 tetrazole to activate the RNA amidite, NH_4OH to remove the exocyclic amino protecting groups, tetra-*n*-butylammonium fluoride (TBAF) to remove the 2'-OH alkylsilyl protecting groups, and gel purification and analysis of the deprotected RNA. In particular this applies to, but is not limited to, a certain class of RNA molecules, ribozymes. These may be formed either chemically
10 or using enzymatic methods. Examples of the chemical synthesis, deprotection, purification and analysis procedures are provided by Usman et al., 1987 *J. American Chem. Soc.*, 109, 7845, Scaringe et al. *Nucleic Acids Res.* 1990, 18, 5433-5341, Perreault et al. *Biochemistry* 1991, 30 4020-4025, and Slim and Gait *Nucleic Acids Res.* 1991, 19, 1183-1188. Odai et al. *FEBS*
15 *Lett.* 1990, 267, 150-152 describes a reverse phase chromatographic purification of RNA fragments used to form a ribozyme. All the above noted references are all hereby incorporated by reference herein.

The aforementioned chemical synthesis, deprotection, purification and analysis procedures are time consuming (10-15 m coupling times) and may
20 also be affected by inefficient activation of the RNA amidites by tetrazole, time consuming (6-24 h) and incomplete deprotection of the exocyclic amino protecting groups by NH_4OH , time consuming (6-24 h), incomplete and difficult to desalt TBAF-catalyzed removal of the alkylsilyl protecting groups, time consuming and low capacity purification of the RNA by gel
25 electrophoresis, and low resolution analysis of the RNA by gel electrophoresis.

Imazawa and Eckstein, 1979 *J. Org. Chem.*, 12, 2039, describe the synthesis of 2'-amino-2'-deoxyribofuranosyl purines. They state that—

30 "To protect the 2'-amino function, we selected the trifluoroacetyl group which can easily be removed."

Chemical linkage

Jennings et al., US Patent No. 5,298,612 describe the use of non-nucleotides to assemble a hammerhead ribozyme lacking a stem II portion.

Draper et al., WO 93/23569 (PCT/US93/04020) describes synthesis of
5 ribozymes in two parts in order to aid in the synthetic process (see, e.g., p. 40).

Usman et al., WO 95/06731, describe enzymatic nucleic acid molecules having non-nucleotides within their structure. Such non-nucleotides can be used in place of nucleotides to allow formation of an enzymatic nucleic acid.

This invention relates to improved methods for synthesis of enzymatic
10 nucleic acids and, in particular, hammerhead and hairpin motif ribozymes. This invention is advantageous over iterative chemical synthesis of ribozymes since the yield of the final ribozyme can be significantly increased. Rather than synthesizing, for example, a 37mer hammerhead ribozyme, two partial ribozyme portions, e.g., a 20mer and a 17mer, can be synthesized in
15 significantly higher yield, and the two reacted together to form the desired enzymatic nucleic acid.

Referring to Fig. 68, the strategy involved is shown for a hammerhead ribozyme where each n or n' is independently any desired nucleotide or non-nucleotide, each filled-in circle represents pairing between bases or other
20 entities, and the solid line represents a covalent bond. Within the structure each n and n' may be a ribonucleotide, a 2'-methoxy-substituted nucleotide, or any other type of nucleotide which does not significantly affect the desired enzymatic activity of the final product (see Usman et al., supra). In the particular embodiment shown, which is not limiting in this invention, five
25 ribonucleotides are provided at rG5, rA6, rG8, rG12, and rA15.1. U4 and U7 may be abasic (i.e., lacking the uridine moiety) or may be ribonucleotides, 2'-methoxy substituted nucleotides, or other such nucleotides. a9, a13, and a14 are preferably 2'-methoxy or may have other substituents. The synthesis of this hammerhead ribozyme is performed by synthesizing a 3' and a 5' portion
30 as shown in a lower part of Fig. 68. Each 5' and 3' portion has a chemically reactive group X and Y, respectively. Non-limiting examples of such chemically reactive groups are provided in Fig. 69. These groups undergo

chemical reactions to provide the bonds shown in Fig. 69. Thus, the X and Y can be used, in various combinations, in this invention to form a chemical linkage between two ribozyme portions.

Thus, the invention features a method for synthesis of an enzymatically
5 active nucleic acid (as defined by Draper, supra) by providing a 3' and a 5' portion of that nucleic acid, each having independently chemically reactive groups at the 5' and 3' positions, respectively. The reaction is performed under conditions in which a covalent bond is formed between the 3' and 5' portions by those chemically reactive groups. The bond formed can be, but is
10 not limited to, either a disulfide, morpholino, amide, ether, thioether, amine, a double bond, a sulfonamide, carbonate, hydrazone or ester bond. The bond is not the natural bond formed between a 5' phosphate group and a 3' hydroxyl group which is made during normal synthesis of an oligonucleotide. In other embodiments, more than two portions can be linked together using
15 pairs of X and Y groups which allow proper formation of the ribozyme (see Figure 69).

By "chemically reactive group" is simply meant a group which can react with another group to form the desired bonds. These bonds may be formed under any conditions which will not significantly affect the structure of the
20 resulting enzymatic nucleic acid. Those in the art will recognize that suitable protecting groups can be provided on the ribozyme portions.

In preferred embodiments the nucleic acid has a hammerhead motif and the 3' and 5' portions each have chemically reactive groups in or immediately adjacent to the stem II region (see Fig. 1). The stem II region is evident in Fig.
25 1 between the bases termed a9 and rG12. The C and G within this stem defines the end of the stem II region. Thus, any of the n or n' moieties within the stem II region can be provided with a chemically reactive group. As is evident from this structure, the chemically reactive groups need not be provided in the solid line portion but can be provided at any of the n or n'. In
30 this way the length of each of the 5' and 3' portions can vary by several bases (Figure 70).

In other preferred embodiments, the chemically reactive group can be, but is not limited to, $(\text{CH}_2)_n\text{SH}$; $(\text{CH}_2)_n\text{NHR}$; $(\text{CH}_2)_n\text{X}$; ribose; COOH ; $(\text{CH}_2)_n\text{PPh}_3$; $(\text{CH}_2)_n\text{SO}_2\text{Cl}$; $(\text{CH}_2)_n\text{COR}$; $(\text{CH}_2)_n\text{RNH}$ or $(\text{CH}_2)_n\text{OH}$, where, CH_2 can be replaced by another group which forms a linking chain (which does not interfere with the terminal chemically reactive group) containing various atoms including, but not limited to CH_2 , such as methylenes, ether, ethylene glycol, thioethers, double bonds, aromatic groups and others, generally at most 20 such atoms are provided in the linking chain, most preferably only 5 - 10 atoms, and even more preferably only 3- 5 atoms; each n independently is an integer from 0 to 10 inclusive and may be the same or different; each R independently is a proton or an alkyl, alkenyl (as described above) and other functional groups or conjugates such as peptides, steroids, hormones, lipids, nucleic acid sequences and others that provides nuclease resistance, improved cell association, improved cellular uptake or interacellular localization. X is halogen, and Ph represents a phenyl ring.

In yet other preferred embodiments, the conditions include provision of NaIO_4 in contact with the ribose, and subsequent provision of a reducing group such as NaBH_4 or NaCNBH_3 ; or the conditions include provision of a coupling reagent.

In a second related aspect, the invention features a mixture of the 5' and 3' portions of the enzymatically active nucleic acids having the 3' and 5' chemically reactive groups noted above.

Those in the art will recognize that while examples are provided of half ribozymes it is possible to provide ribozymes in 3 or more portions. For example, the hairpin ribozyme may be synthesized by inclusion of chemically reactive groups in helix IV and in other helices which are not critical to the enzymatic activity of the nucleic acid.

Pol III-based vectors

This invention relates to RNA polymerase III-based methods and systems for expression of therapeutic RNAs in cells *in vivo* or *in vitro*.

The RNA polymerase III (pol III) promoter is one found in DNA encoding 5S, U6, adenovirus VA1, Vault, telomerase RNA, tRNA genes, etc., and is transcribed by RNA polymerase III (for a review see Geiduschek and Tocchini-Valentini, 1988 *Annu. Rev. Biochem.* 57, 873-914; Willis, 1993 *Eur. J. Biochem.* 212, 1-11). There are three major types of pol III promoters: types 1, 2 and 3 (Geiduschek and Tocchini-Valentini, 1988 *supra*; Willis, 1993 *supra*) (see Figure 1). Type 1 pol III promoter consists of three cis-acting sequence elements downstream of the transcriptional start site a) 5' sequence element (A block); b) an intermediate sequence element (I block); c) 3' sequence element (C block). 5S ribosomal RNA genes are transcribed using the type 1 pol III promoter (Specht et al., 1991 *Nucleic Acids Res.* 19, 2189-2191).

The type 2 pol III promoter is characterized by the presence of two cis-acting sequence elements downstream of the transcription start site. All Transfer RNA (tRNA), adenovirus VA RNA and Vault RNA (Kikhoefer et al., 1993, *J. Biol. Chem.* 268, 7868-7873) genes are transcribed using this promoter (Geiduschek and Tocchini-Valentini, 1988 *supra*; Willis, 1993 *supra*). The sequence composition and orientation of the two cis-acting sequence elements- A box (5' sequence element) and B box (3' sequence element) are essential for optimal transcription by RNA polymerase III.

The type 3 pol III promoter contains all of the cis-acting promoter elements upstream of the transcription start site. Upstream sequence elements include a traditional TATA box (Mattaj et al., 1988 *Cell* 55, 435-442), proximal sequence element (PSE) and a distal sequence element (DSE; Gupta and Reddy, 1991 *Nucleic Acids Res.* 19, 2073-2075). Examples of genes under the control of the type 3 pol III promoter are U6 small nuclear RNA (U6 snRNA) and Telomerase RNA genes.

In addition to the three predominant types of pol III promoters described above, several other pol III promoter elements have been reported (Willis, 1993 *supra*) (see Figure 76). Epstein-Barr-virus-encoded RNAs (EBER), *Xenopus* seleno-cysteine tRNA and human 7SL RNA are examples of genes that are under the control of pol III promoters distinct from the aforementioned types of promoters. EBER genes contain a functional A and B box (similar to type 2 pol III promoter). In addition they also require an EBER-specific TATA

box and binding sites for ATF transcription factors (Howe and Shu, 1989 *Cell* 57,825-834). The seleno-cysteine tRNA gene contains a TATA box, PSE and DSE (similar to type 3 pol III promoter). Unlike most tRNA genes, the seleno-cysteine tRNA gene lacks a functional A box sequence element. It does
5 require a functional B box (Lee et al., 1989 *J. Biol. Chem.* 264, 9696-9702). The human 7SL RNA gene contains an unique sequence element downstream of the transcriptional start site. Additionally, upstream of the transcriptional start site, the 7SL gene contains binding sites for ATF class of transcription factors and a DSE (Bredow et al., 1989 *Gene* 86, 217-225).

10 Gilboa WO 89/11539 and Gilboa and Sullenger WO 90/13641 describe transformation of eucaryotic cells with DNA under the control of a pol III promoter. They state:

 "In an attempt to improve antisense RNA synthesis using stable gene transfer
15 protocols, the use of pol III promoters to drive the expression of antisense RNA can be considered. The underlying rationale for the use of pol III promoters is that they can generate substantially higher levels of RNA transcripts in cells as compared to pol II promoters. For example, it is estimated that in a eucaryotic cell there are about 6×10^7 t-RNA molecules and 7×10^5 mRNA molecules, i.e., about 100 fold more pol III transcripts of this class than total pol II transcripts. Since there are about 100 active t-RNA genes
20 per cell, each t-RNA gene will generate on the average RNA transcripts equal in number to total pol II transcripts. Since an abundant pol II gene transcript represents about 1% of total mRNA while an average pol II transcript represents about 0.01% of total mRNA, a t-RNA (pol III) based transcriptional unit may be able to generate 100 fold to 10,000 fold more RNA than a pol II based transcriptional unit. Several reports have described the use of pol III promoters to express RNA in eucaryotic cells. Lewis and Manley and
25 Sisodia have fused the Adenovirus VA-1 promoter to various DNA sequences (the herpes TK gene, globin and tubulin) and used transfection protocols to transfer the resulting DNA constructs into cultured cells which resulted in transient synthesis of RNA in the transduced cell. De la Pena and Zasloff have expressed a t-RNA-Herpes TK fusion DNA construct upon microinjection into frog oocytes. Jennings and Molloy have
30 constructed an antisense RNA template by fusing the VA-1 gene promoter to a DNA fragment derived from SV40 based vector which also resulted in transient expression of antisense RNA and limited inhibition of the target gene". [Citations omitted.]

The authors describe a fusion product of a chimeric tRNA and an RNA product (see Fig. 1C of WO 90/13641). In particular they describe a human tRNA_{met} derivative 3-5. 3-5 was derived from a cloned human tRNA gene by deleting 19 nucleotides from the 3' end of the gene. The authors indicate that
5 the truncated gene can be transcribed if a termination signal is provided, but that no processing of the 3' end of the RNA transcript takes place.

Adeniyi-Jones et al., 1984 *Nucleic Acids Res.* 12, 1101-1115, describe certain constructions which "may serve as the basis for utilizing the tRNA gene as a 'portable promoter' in engineered genetic constructions." The authors
10 describe the production of a so-called $\Delta 3'-5$ in which 11 nucleotides of the 3'-end of the mature tRNA_{met} sequence are replaced by a plasmid sequence, and are not processed to generate a mature tRNA. The authors state:

"the properties of the tRNA_{met} 3' deletion plasmids described in this study suggest their potential use in certain engineered genetic constructions. The tRNA gene could
15 be used to promote transcription of theoretically any DNA sequence fused to the 3' border of the gene, generating a fusion gene which would utilize the efficient polymerase III promoter of the human tRNA_{met} gene. By fusion of the DNA sequence to a tRNA_{met} deletion mutant such as $\Delta 3'-4$, a long read-through transcript would be generated in vivo (dependent, of course, on the absence of effective RNA polymerase
20 III termination sequences). Fusion of the DNA sequence to a tRNA_{met} deletion mutant such as $\Delta 3'-5$ would lead to the generation of a co-transcript from which subsequent processing of the tRNA leader at the 5' portion of the fused transcript would be blocked. Control over processing may be of some biological use in engineered constructions, as suggested by properties of mRNA species bearing tRNA sequences as 5' leaders in
25 prokaryotes. Such "dual transcripts" code for several predominant bacterial proteins such as EF-Tu and may use the tRNA leaders as a means of stabilizing the transcript from degradation in vivo. The potential use of the tRNA_{met} gene as a "promoter leader" in eukaryotic systems has been realized recently in our laboratory. Fusion genes consisting of the deleted tRNA_{met} sequences contained on plasmids $\Delta 3'-4$
30 and $\Delta 3'-5$ in front of a promoter-less Herpes simplex type I thymidine kinase gene yield viral-specific enzyme resulting from RNA polymerase III dependent transcription in both *X. laevis* oocytes and somatic cells". [References omitted].

Sullenger et al., 1990 *Cell* 63, 601-619, describe over-expression of *TAR*-containing sequences using a chimeric tRNA^{met}-*TAR* transcription unit in a double copy (DC) murine retroviral vector.

5 Sullenger et al., 1990 *Molecular and Cellular Bio.* 10, 6512, describe expression of chimeric tRNA driven antisense transcripts. It indicates:

10 "successful use of a tRNA-driven antisense RNA transcription system was dependent on the use of a particular type of retroviral vector, the double-copy (DC) vector, in which the chimeric tRNA gene was inserted in the viral LTR. The use of an RNA pol III-based transcription system to stably express high levels of foreign RNA sequences in cells
15 may have other important applications. Foremost, it may significantly improve the ability to inhibit endogenous genes in eucaryotic cells for the study of gene expression and function, whether antisense RNA, ribozymes, or competitors of sequence-specific binding factors are used. tRNA-driven transcription systems may be particularly useful for introducing "mutations" into the germ line, i.e., for generating transgenic animals or
20 transgenic plants. Since tRNA genes are ubiquitously expressed in all cell types, the chimeric tRNA genes may be properly expressed in all tissues of the animal, in contrast to the more idiosyncratic behavior of RNA pol II-based transcription units. However, homologous recombination represents a more elegant although, at present, very cumbersome approach for introducing mutations into the germ line. In either case, the
25 ability to generate transgenic animals or plants carrying defined mutations will be an extremely valuable experimental tool for studying gene function in a developmental context and for generating animal models for human genetic disorders. In addition, tRNA-driven gene inhibition strategies may also be useful in creating pathogen-resistant livestock and plants. [References omitted.]

25 Cotten and Birnstiel, 1989 *EMBO Jnl.* 8, 3861, describe the use of tRNA genes to increase intracellular levels of ribozymes. The authors indicate that the ribozyme coding sequences were placed between the A and the B box internal promoter sequences of the *Xenopus* tRNA^{met} gene. They also indicate that the targeted hammerhead ribozymes were active *in vivo*.

30 Yu et al., 1993 *Proc. Natl. Acad. Sci. USA* 90, 5340, describe the use of a VAI promoter to express a hairpin ribozyme. The resulting transcript consisted

of the first 104 nucleotides of the VAI RNA, followed by the ribozyme sequence and the terminator sequence.

Lieber and Strauss, 1995 *Mol. Cellular Bio.* 15, 540, inserted a hammerhead ribozyme sequence in the central domain of a VAI RNA.

- 5 Pol III-based vectors are described in Stinchcomb et al., WO 95/23225. Another example is provided below.

Example 1: Stromelysin Hammerhead ribozymes

- By engineering ribozyme motifs applicant has designed several ribozymes directed against stromelysin mRNA sequences. These ribozymes
10 are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave stromelysin target sequences *in vitro* is evaluated.

- The ribozymes are tested for function *in vivo* by analyzing stromelysin expression levels. Ribozymes are delivered to cells by incorporation into
15 liposomes, by complexing with cationic lipids, by microinjection, and/or by expression from DNA/RNA vectors. Stromelysin expression is monitored by biological assays, ELISA, by indirect immunofluoresence, and/or by FACS analysis. Stromelysin mRNA levels are assessed by Northern analysis, RNase protection, primer extension analysis and/or quantitative RT-PCR.
20 Ribozymes that block the induction of stromelysin activity and/or stromelysin mRNA by more than 50% are identified.

- Ribozymes targeting selected regions of mRNA associated with arthritic disease are chosen to cleave the target RNA in a manner which preferably inhibits translation of the RNA. Genes are selected such that inhibition of
25 translation will preferably inhibit cell replication, e.g., by inhibiting production of a necessary protein or prevent production of an undesired protein, e.g., stromelysin. Selection of effective target sites within these critical regions of mRNA may entail testing the accessibility of the target RNA to hybridization with various oligonucleotide probes. These studies can be performed using
30 RNA or DNA probes and assaying accessibility by cleaving the hybrid molecule with RNaseH (see below). Alternatively, such a study can use

ribozyme probes designed from secondary structure predictions of the mRNAs, and assaying cleavage products by polyacrylamide gel electrophoresis (PAGE), to detect the presence of cleaved and uncleaved molecules.

5 In addition, potential ribozyme target sites within the rabbit stromelysin mRNA sequence (1795 nucleotides) were located and aligned with the human target sites. Because the rabbit stromelysin mRNA sequence has an 84% sequence identity with the human sequence, many ribozyme target sites are also homologous. Thus, the rabbit has potential as an appropriate animal
10 model in which to test ribozymes that are targeted to human stromelysin but have homologous or nearly homologous cleavage sites on rabbit stromelysin mRNA as well (Tables AII-AVI, AVIII & AIX). Thirty of the 316 UH sites in the rabbit sequence are identical with the corresponding site in the human sequence with respect to at least 14 nucleotides surrounding the potential
15 ribozyme cleavage sites. The nucleotide in the RNA substrate that is immediately adjacent (5') to the cleavage site is unpaired in the ribozyme-substrate complex (see Fig. 1) and is consequently not included in the comparison of human and rabbit potential ribozyme sites. In choosing human ribozyme target sites for continued testing, the presence of identical or nearly
20 identical sites in the rabbit sequence is considered.

Example 2: Superior sites

Potential ribozyme target sites were subjected to further analysis using computer folding programs (Mfold or a Macintosh-based version of the following program, LRNA (Zucker (1989) Science 244:48), to determine if 1)
25 the target site is substantially single-stranded and therefore predicted to be available for interaction with a ribozyme, 2) if a ribozyme designed to that site is predicted to form stem II but is generally devoid of any other intramolecular base pairing, and 3) if the potential ribozyme and the sequence flanking both
30 sides of the cleavage site together are predicted to interact correctly. The sequence of Stem II can be altered to maintain a stem at that position but minimize intramolecular basepairing with the ribozyme's substrate binding arms. Based on these minimal criteria, and including all the sites that are identical in human and rabbit stromelysin mRNA sequence, a subset of 66

potential superior ribozyme target sites was chosen (as first round targets) for continued analysis. These are SEQ. ID. NOS.: 34, 35, 37, 47, 54, 57, 61, 63, 64, 66, 76, 77, 79, 87, 88, 96, 97, 98, 99, 100, 107, 110, 121, 126, 128, 129, 133, 140, 146, 148, 151, 162, 170, 179, 188, 192, 194, 196, 199, 202, 203, 207, 208, 218, 220, 223, 224, 225, 227, 230, 232, 236, 240, 245, 246, 256, 259, 260, 269, 280, 281, 290, 302, 328, 335 and 353 (see Table AIII).

Example 3: Accessible sites

To determine if any or all of these potential superior sites might be accessible to a ribozyme directed to that site, an RNase H assay is carried out. Using this assay, the accessibility of a potential ribozyme target site to a DNA oligonucleotide probe can be assessed without having to synthesize a ribozyme to that particular site. If the complementary DNA oligonucleotide is able to hybridize to the potential ribozyme target site then RNase H, which has the ability to cleave the RNA of a DNA/RNA hybrid, will be able to cleave the target RNA at that particular site. Specific cleavage of the target RNA by RNase H is an indication that that site is "open" or "accessible" to oligonucleotide binding and thus predicts that the site will also be open for ribozyme binding. By comparing the relative amount of specific RNase H cleavage products that are generated for each DNA oligonucleotide/site, potential ribozyme sites can be ranked according to accessibility.

To analyze target sites using the RNase H assay, DNA oligonucleotides (generally 13-15 nucleotides in length) that are complementary to the potential target sites are synthesized. Body-labeled substrate RNAs (either full-length RNAs or ~500-600 nucleotide subfragments of the entire RNA) are prepared by *in vitro* transcription in the presence of a ^{32}P -labeled nucleotide. Unincorporated nucleotides are removed from the ^{32}P -labeled substrate RNA by spin chromatography on a G-50 Sephadex column and used without further purification. To carry out the assay, the ^{32}P -labeled substrate RNA is pre-incubated with the specific DNA oligonucleotide (1 μM and 0.1 μM final concentration) in 20 mM Tris-HCl, pH 7.9, 100 mM KCl, 10 mM MgCl_2 , 0.1 mM EDTA, 0.1 mM DTT at 37°C for 5 minutes. An excess of RNase H (0.8 units/10 μl reaction) is added and the incubation is continued for 10 minutes. The reaction is quenched by the addition of an equal volume of 95% formamide,

20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. RNase H-cleaved RNA products are separated from uncleaved RNA on denaturing polyacrylamide gels, visualized
5 by autoradiography and the amount of cleavage product is quantified.

RNase H analysis on the 66 potential ribozyme sites (round 1) was carried out and those DNA oligonucleotides/sites that supported the most RNase H cleavage were determined. These assays were carried out using full-length human and rabbit stromelysin RNA as substrates. Results
10 determined on human stromelysin RNA indicated that 23 of the 66 sites supported a high level of RNase H cleavage, and an additional 13 supported a moderate level of RNase H cleavage. Twenty-two sites were chosen from among these two groups for continued study. Two of the criteria used for making this choice were 1) that the particular site supported at least moderate
15 RNase H cleavage on human stromelysin RNA and 2) that the site have two or fewer nucleotide differences between the rabbit and the human stromelysin sequence. RNase H accessibility on rabbit stromelysin RNA was determined, but was not used as a specific criteria for these choices. Those DNA oligonucleotides that are not totally complementary to the rabbit sequence
20 may not be good indicators of the relative amount of RNase H cleavage, possibly because the mismatch leads to less efficient hybridization of the DNA oligonucleotide to the mismatched RNA substrate and therefore less RNase H cleavage is seen.

Example 4: Analysis of Ribozymes

25 Ribozymes were then synthesized to 22 sites (Table AV) predicted to be accessible as judged the RNase H assay. Eleven of these 22 sites are identical to the corresponding rabbit sites. The 22 sites are SEQ. ID, NOS.: 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, 281. The 22 ribozymes were chemically synthesized
30 with recognition arms of either 7 nucleotides or 8 nucleotides, depending on which ribozyme alone and ribozyme-substrate combinations were predicted by the computer folding program (MulFold) to fold most correctly. After synthesis, ribozymes are either purified by HPLC or gel purified.

These 22 ribozymes were then tested for their ability to cleave both human and rabbit full-length stromelysin RNA. Full-length, body-labeled stromelysin RNA is prepared by *in vitro* transcription in the presence of [α - 32 P]CTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further purification. Assays are performed by prewarming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl₂) and the cleavage reaction is initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that has also been prewarmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of 1 μ M and 0.1 μ M ribozyme, *i.e.*, ribozyme excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Full-length substrate RNA and the specific RNA products generated by ribozyme cleavage are visualized on an autoradiograph of the gel.

Of the 22 ribozymes tested, 21 were able to cleave human and rabbit substrate RNA *in vitro* in a site-specific manner. In all cases, RNA cleavage products of the appropriate lengths were visualized. The size of the RNA was judged by comparison to molecular weight standards electrophoresed in adjacent lanes of the gel. The fraction of substrate RNA cleaved during a ribozyme reaction can be used as an assessment of the activity of that ribozyme *in vitro*. The activity of these 22 ribozymes on full-length substrate RNA ranged from approximately 10% to greater than 95% of the substrate RNA cleaved in the ribozyme cleavage assay using 1 μ M ribozyme as described above. A subset of seven of these ribozymes was chosen for continued study. These seven ribozymes (denoted in Table AV) were among those with the highest activity on both human and rabbit stromelysin RNA. Five of these seven sites have sequence identity between human and rabbit stromelysin RNAs for a minimum of 7 nucleotides in both directions flanking the cleavage site. These sites are 883, 947, 1132, 1221 and 1410. and the ribozymes are SEQ. ID. NOS.: 368, 369, 370, 371, 372, 373, and 374.

Example 5: Arm Length Tests

In order to test the effect of arm length variations on the cleavage activity of a ribozyme to a particular site *in vitro*, ribozymes to these seven sites were designed that had alterations in the binding arm lengths. For each site, a complete set of ribozymes was synthesized that included ribozymes with binding arms of 6 nucleotides, 7 nucleotides, 8 nucleotides, 10 nucleotides and 12 nucleotides, i.e., 5 ribozymes to each site. These ribozymes were gel-purified after synthesis and tested in ribozyme cleavage assays as described above.

After analysis of the 35 ribozymes, five ribozymes with varied arm lengths to each of these seven sites, it was clear that two ribozymes were the most active *in vitro*. These two ribozymes had seven nucleotide arms directed against human sequence cleavage sites of nucleotide 617 and nucleotide 820. These are referred to as RZ 617H 7/7 and RZ 820H 7/7 denoting the human (H) sequence cleavage site (617 or 820) and the arm length on the 5' and 3' side of the ribozyme molecule.

Example 6: Testing the efficacy of ribozymes in cell culture

The two most active ribozymes *in vitro* (RZ 617H 7/7 and RZ 820H 7/7) were then tested for their ability to cleave stromelysin mRNA in the cell. Primary cultures of human or rabbit synovial fibroblasts were used in these experiments. For these efficacy tests, ribozymes with 7 nucleotide arms were synthesized with 2' O- methyl modifications on the 5 nucleotides at the 5' end of the molecule and on the 5 nucleotides at the 3' end of the molecule. For comparison, ribozymes to the same sites but with 12 nucleotide arms (RZ 617H 12/12 and RZ 820H 12/12) were also synthesized with the 2' O methyl modifications at the 5 positions at the end of both binding arms. Inactive ribozymes that contain 2 nucleotide changes in the catalytic core region were also prepared for use as controls. The catalytic core in the inactive ribozymes is C U U A U G A G G C C G A A A G G C C G A U versus CUGAUGAGGCCGAAAGGCCGAA in the active ribozymes. The inactive ribozymes show no cleavage activity *in vitro* when measured on full-length RNA in the typical ribozyme cleavage assay at a 1 μ M concentration for 1 hour.

The general assay was as follows: Fibroblasts, which produce stromelysin, are serum-starved overnight and ribozymes or controls are offered to the cells the next day. Cells are maintained in serum-free media. The ribozyme can be applied to the cells as free ribozyme, or in association
5 with various delivery vehicles such as cationic lipids (including Transfectam™, Lipofectin™ and Lipofectamine™), conventional liposomes, non-phospholipid liposomes or biodegradable polymers. At the time of ribozyme addition, or up to 3 hours later, Interleukin-1 α (typically 20 units/ml) can be added to the cells to induce a large increase in stromelysin expression. The production of
10 stromelysin can then be monitored over a time course, usually up to 24 hours.

If a ribozyme is effective in cleaving stromelysin mRNA within a cell, the amount of stromelysin mRNA will be decreased or eliminated. A decrease in the level of cellular stromelysin mRNA, as well as the appearance of the RNA products generated by ribozyme cleavage of the full-length stromelysin mRNA,
15 can be analyzed by methods such as Northern blot analysis, RNase protection assays and/or primer extension assays. The effect of ribozyme cleavage of cellular stromelysin mRNA on the production of the stromelysin protein can also be measured by a number of assays. These include the ELISA (Enzyme-Linked Immuno Sorbent Assay) and an immunofluorescence assay described
20 below. In addition, functional assays have been published that monitor stromelysin's enzymatic activity by measuring degradation of its primary substrate, proteoglycan.

Example 7: Analysis of Stromelysin Protein

Stromelysin secreted into the media of Interleukin-1 α -induced human
25 synovial fibroblasts was measured by ELISA using an antibody that recognizes human stromelysin. Where present, a Transfectam™-ribozyme complex (0.15 μ M ribozyme final concentration) was offered to 2-4 x 10⁵ serum-starved cells for 3 hours prior to induction with Interleukin-1 α . The Transfectam™ was prepared according to the manufacturer (Promega Corp.)
30 except that 1:1 (w/w) dioleoyl phosphatidylethanolamine was included. The Transfectam™-ribozyme complex was prepared in a 5:1 charge ratio. Media was harvested 24 hours after the addition of Interleukin-1 α . The control (NO RZ) is Transfectam™ alone applied to the cell. Inactive ribozymes, with 7

- nucleotide arms or 12 nucleotide arms have the two inactivating changes to the catalytic core that are described above. Cell samples were prepared in duplicate and the assay was carried out on several dilutions of the conditioned media from each sample. Results of the ELISA are presented below as a percent of stromelysin present vs. the control (NO RZ) which is set at 100%.

		<u>RZ TARGET SITE</u>	
<u>TREATMENT</u>		<u>617H</u>	<u>820H</u>
	RZ 7/7	06.83	07.05
	RZ 12/12	18.47	33.90
10	INACTIVE RZ 7/7	100	100
	INACTIVE RZ 12/12	100	100
	NO RZ CONTROL	100	100

- The results above clearly indicate that treatment with active ribozyme, either RZ 617H 7/7 and RZ 820H 7/7, has a dramatic effect on the amount of stromelysin secreted by the cells. When compared to untreated, control cells or cells treated with inactive ribozymes, the level of stromelysin was decreased by approximately 93%. Ribozymes to the same sites, but synthesized with 12 nucleotide binding arms, were also efficacious, causing a decrease in stromelysin to ~66 to ~81% of the control. In previous *in vitro* ribozyme cleavage assays, RZ 617H 7/7 and RZ 820H 7/7 had better cleavage activity on full-length RNA substrates than ribozymes with 12 nucleotide arms directed to the same sites (617H 12/12 and RZ 820H 12/12).

25 Example 8: Immunofluorescent Assay

An alternative method of stromelysin detection is to visualize stromelysin protein in the cells by immunofluorescence. For this assay, cells are treated

with monensin to prevent protein secretion from the cell. The stromelysin retained by the cells after monensin addition can then be visualized by immunofluorescence using either conventional or confocal microscopy. Generally, cells were serum-starved overnight and treated with ribozyme the following day for several hours. Monensin was then added and after ~5-6 hours, monensin-treated cells were fixed and permeabilized by standard methods and incubated with an antibody recognizing human stromelysin. Following an additional incubation period with a secondary antibody that is conjugated to a fluorophore, the cells were observed by microscopy. A decrease in the amount of fluorescence in ribozyme-treated cells, compared to cells treated with inactive ribozymes or media alone, indicates that the level of stromelysin protein has been decreased due to ribozyme treatment.

As visualized by the immunofluorescence technique described above, treatment of human synovial fibroblasts with either RZ 617H 7/7 or RZ 820H 7/7 (final concentrations of 1.5 μ M free ribozyme or 0.15 μ M ribozyme complexed with Transfectam™ resulted in a significant decrease in fluorescence, and therefore stromelysin protein, when compared with controls. Controls consisted of treating with media or Transfectam™ alone. Treatment of the cells with the corresponding inactive ribozymes with two inactivating changes in the catalytic core resulted in immunofluorescence similar to the controls without ribozyme treatment.

Rabbit synovial fibroblasts were also treated with RZ 617H 7/7 or RZ 820H 7/7, as well as with the two corresponding ribozymes (RZ 617R 7/7 or RZ 820R 7/7) that each have the appropriate one nucleotide change to make them completely complementary to the rabbit target sequence. Relative to controls that had no ribozyme treatment, immunofluorescence in Interleukin-1 α -induced rabbit synovial fibroblasts was visibly decreased by treatment with these four ribozymes, whether specific for rabbit or human mRNA sequence. For the immunofluorescence study in rabbit synovial fibroblasts, the antibody to human stromelysin was used.

Example 9: Ribozyme Cleavage of Cellular RNA

The following method was used in this example.

Primer extension assay:

The primer extension assay was used to detect full-length RNA as well as the 3' ribozyme cleavage products of the RNA of interest. The method involves synthesizing a DNA primer (generally ~20 nucleotides in length) that
5 can hybridize to a position on the RNA that is downstream (3') of the putative ribozyme cleavage site. Before use, the primer was labeled at the 5' end with ^{32}P [ATP] using T4 polynucleotide kinase and purified from a gel. The labeled primer was then incubated with a population of nucleic acid isolated from a cellular lysate by standard procedures. The reaction buffer was 50 mM Tris-
10 HCl, pH 8.3, 3 mM MgCl_2 , 20 mM KCl, and 10 mM DTT. A 30 minute extension reaction follows, in which all DNA primers that have hybridized to the RNA were substrates for reverse transcriptase, an enzyme that will add nucleotides to the 3' end of the DNA primer using the RNA as a template. Reverse transcriptase was obtained from Life Technologies and is used
15 essentially as suggested by the manufacturer. Optimally, reverse transcriptase will extend the DNA primer, forming cDNA, until the end of the RNA substrate is reached. Thus, for ribozyme-cleaved RNA substrates, the cDNA product will be shorter than the resulting cDNA product of a full-length, or uncleaved RNA substrate. The differences in size of the ^{32}P -labeled
20 cDNAs produced by extension can then be discriminated by electrophoresis on a denaturing polyacrylamide gel and visualized by autoradiography.

Strong secondary structure in the RNA substrate can, however, lead to premature stops by reverse transcriptase. This background of shorter cDNAs is generally not a problem unless one of these prematurely terminated
25 products electrophoreses in the expected position of the ribozyme-cleavage product of interest. Thus, 3' cleavage products are easily identified based on their expected size and their absence from control lanes. Strong stops due to secondary structure in the RNA do, however, cause problems in trying to quantify the total full-length and cleaved RNA present. For this reason, only
30 the relative amount of cleavage can easily be determined.

The primer extension assay was carried out on RNA isolated from cells that had been treated with TransfectamTM-complexed RZ 617H 7/7, RZ 820H 7/7, RZ 617H 12/12 and RZ 820H 12/12. Control cells had been treated with

Transfectam™ alone. Primer extensions on RNA from cells treated with the Transfectam™ complexes of the inactive versions of these four ribozymes were also prepared. The 20 nucleotide primer sequence is 5' AATGAAAACGAGGTCCTTGC 3' and it is complementary to a region about 285 nucleotides downstream of ribozyme site 820. For ribozymes to site 617, the cDNA length for the 3' cleavage product is 488 nucleotides, for 820 the cDNA product is 285 nucleotides. Full-length cDNA will be 1105 nucleotides in length. Where present, 1 ml of 0.15 μ M ribozyme was offered to $\sim 2-3 \times 10^5$ serum-starved human synovial fibroblasts. After 3 hours, 20 units/ml Interleukin-1 α was added to the cells and the incubation continued for 24 hours.

³²P-labeled cDNAs of the correct sizes for the 3' products were clearly visible in lanes that contained RNA from cells that had been treated with active ribozymes to sites 617 and 820. Ribozymes with 7 nucleotide arms were judged to be more active than ribozymes with 12 nucleotide arms by comparison of the relative amount of 3' cleavage product visible. This correlates well with the data obtained by ELISA analysis of the conditioned media from these same samples. In addition, no cDNAs corresponding to the 3' cleavage products were visible following treatment of the cells with any of the inactive ribozymes.

To insure that ribozyme cleavage of the RNA substrate was not occurring during the preparation of the cellular RNA or during the primer extension reaction itself, several controls have been carried out. One control was to add body-labeled stromelysin RNA, prepared by *in vitro* transcription, to the cellular lysate. This lysate was then subjected to the typical RNA preparation and primer extension analysis except that non-radioactive primer was used. If ribozymes that are present in the cell at the time of cell lysis are active under any of the conditions during the subsequent analysis, the added, body-labeled stromelysin RNA will become cleaved. This, however, is not the case. Only full-length RNA was visible by gel analysis, no ribozyme cleavage products were present. This is evidence that the cleavage products detected in RNA from ribozyme-treated cells resulted from ribozyme cleavage in the cell, and not during the subsequent analysis.

Example 10: RNase Protection Assay

By RNase protection analysis, both the 3' and the 5' products generated by ribozyme cleavage of the substrate RNA in a cell can be identified. The RNase protection assay is carried out essentially as described in the protocol provided with the Lysate Ribonuclease Protection Kit (United States Biochemical Corp.) The probe for RNase protection is an RNA that is complementary to the sequence surrounding the ribozyme cleavage site. This "antisense" probe RNA is transcribed *in vitro* from a template prepared by the polymerase chain reaction in which the 5' primer was a DNA oligonucleotide containing the T7 promoter sequence. The probe RNA is body labeled during transcription by including ^{32}P [CTP] in the reaction and purified away from unincorporated nucleotide triphosphates by chromatography on G-50 Sephadex. The probe RNA (100,000 to 250,000 cpm) is allowed to hybridize overnight at 37°C to the RNA from a cellular lysate or to RNA purified from a cell lysate. After hybridization, RNase T₁ and RNase A are added to degrade all single-stranded RNA and the resulting products are analyzed by gel electrophoresis and autoradiography. By this analysis, full-length, uncleaved target RNA will protect the full-length probe. For ribozyme-cleaved target RNAs, only a portion of the probe will be protected from RNase digestion because the cleavage event has occurred in the region to which the probe binds. This results in two protected probe fragments whose size reflects the position at which ribozyme cleavage occurs and whose sizes add up to the size of the full-length protected probe.

RNase protection analysis was carried out on cellular RNA isolated from rabbit synovial fibroblasts that had been treated either with active or inactive ribozyme. The ribozymes tested had 7 nucleotide arms specific to the rabbit sequence but corresponding to human ribozyme sites 617 and 820 (i.e. RZ 617R 7/7, RZ 820R 7/7). The inactive ribozymes to the same sites also had 7 nucleotide arms and included the two inactivating changes described above. The inactive ribozymes were not active on full-length rabbit stromelysin RNA in a typical 1 hour ribozyme cleavage reaction *in vitro* at a concentration of 1 μM. For all samples, one ml of 0.15 μM ribozyme was administered as a Transfectam™ complex to serum-starved cells. Addition of Interleukin-1α followed 3 hours later and cells were harvested after 24 hours. For samples

from cells treated with either active ribozyme tested, the appropriately-sized probe fragments representing ribozyme cleavage products were visible. For site 617, two fragments corresponding to 125 and 297 nucleotides were present, for site 820 the two fragments were 328 and 94 nucleotides in length.

- 5 No protected probe fragments representing RNA cleavage products were visible in RNA samples from cells that not been treated with any ribozyme, or in cells that had received the inactive ribozymes. Full-length protected probe (422 nucleotides in length) was however visible, indicating the presence of full-length, uncleaved stromelysin RNA in these samples.

10 Delivery of Free and Transfectam-Complexed Ribozymes to Fibroblasts

- Ribozymes can be delivered to fibroblasts complexed to a cationic lipid or in free form. To deliver free ribozyme, an appropriate dilution of stock ribozyme (final concentration is usually 1.5 μM) is made in serum-free medium; if a radioactive tracer is to be used (i.e., ^{32}P), the specific activity of
- 15 the ribozyme is adjusted to 800-1200 cpm/pmol. To deliver ribozyme complexed with the cationic lipid Transfectam, the lipid is first prepared as a stock solution containing 1/1 (w/w) dioleoylphosphatidylcholine (DOPE). Ribozyme is mixed with the Transfectam/DOPE mixture at a 1/5 (RZ/TF) charge ratio; for a 36-mer ribozyme, this is a 45-fold molar excess of Transfectam
- 20 (Transfectam has 4 positive charges per molecule). After a 10 min incubation at room temperature, the mixture is diluted and applied to cells, generally at a ribozyme concentration of 0.15 μM . For ^{32}P experiments, the specific activity of the ribozyme is the same as for the free ribozyme experiments.

- After 24 hour, about 30% of the offered Transfectam-ribozyme cpm's are
- 25 cell-associated (in a nuclease-resistant manner). Of this, about 10-15% of the cpm's represent intact ribozyme; this is about 20-25 million ribozymes per cell. For the free ribozyme, about 0.6% of the offered dose is cell-associated after 24 hours. Of this, about 10-15% is intact; this is about 0.6-0.8 million ribozymes per cell.

30 Example 11: *In vitro* cleavage of stromelysin mRNA by HH ribozymes

In order to screen for additional HH ribozyme cleavage sites, ribozymes, targeted against some of the sites listed in example 2 and Table 3, were

synthesized. These ribozymes were extensively modified such that: 5' terminal nucleotides contain phosphorothioate substitutions; except for five ribose residues in the catalytic core, all the other 2'-hydroxyl groups within the ribozyme were substituted with either 2'-O-methyl groups or 2'-C-allyl modifications. The aforementioned modifications are meant to be non-limiting modifications. Those skilled in the art will recognize that other embodiments can be readily generated using the techniques known in the art.

These ribozymes were tested for their ability to cleave RNA substrates *in vitro*. Referring to Fig. 7, *in vitro* RNA cleavage by HH ribozymes targeted to sites 21, 463, 1049, 1366, 1403, 1410 and 1489 (SEQ. ID. NOS. 35, 98, 202, 263, 279, 281 and 292 respectively) was assayed at 37°C. Substrate RNAs were 5' end-labeled using [γ -³²P]ATP and T4 polynucleotide kinase enzyme. In a standard cleavage reaction under "ribozyme excess" conditions, ~1 nM substrate RNA and 40 nM ribozyme were denatured separately by heating to 90°C for 2 min followed by snap cooling on ice for 10 min. The substrate and the ribozyme reaction mixtures were renatured in a buffer containing 50 mM Tris-HCl, pH 7.5 and 10 mM MgCl₂ at 37°C for 10 min. Cleavage reaction was initiated by mixing the ribozyme and the substrate RNA and incubating at 37°C. Aliquots of 5 μ l were taken at regular intervals of time and the reaction quenched by mixing with an equal volume of formamide stop mix. The samples were resolved on a 20% polyacrylamide/urea gel.

A plot of percent RNA substrate cleaved as a function of time is shown in Fig. 7. The plot shows that all six HH ribozymes cleaved the target RNA efficiently. Some HH ribozymes were, however, more efficient than others (e.g., 1049HH cleaves faster than 1366HH).

Ribozyme Efficacy Assay in Cultured HS-27 Cells (Used in the Following Examples):

Ribozymes were assayed on either human foreskin fibroblasts(HS-27) cell line or primary human synovial fibroblasts (HSF). All cells were plated the day before the assay in media containing 10% fetal bovine serum in 24 well plates at a density of 5×10^4 cells/well. At 24 hours after plating, the media was removed from the wells and the monolayers were washed with Dulbeccos

phosphate buffered saline (PBS). The cells were serum starved for 24 h by incubating the cells in media containing 0.5% fetal bovine serum (FBS; 1 ml/well). Ribozyme/lipid complexes were prepared as follows: Ribozymes and LipofectAMINE were diluted separately in serum-free DMEM plus 20 mM Hepes pH 7.3 to 2X final concentration, then equal volumes were combined, vortexed and incubated at 37°C for 15 minutes. The charge ratio of LipofectAmine: ribozyme was 3:1. Cells were washed twice with PBS containing Ca^{2+} and Mg^{2+} . Cells were then treated the ribozyme/lipid complexes and incubated at 37°C for 1.5 hours. FBS was then added to a final concentration of 10%. Two hours after FBS addition, the ribozyme containing solution was removed and 0.5 ml DMEM containing 50 u/ml IL-1, 10% FBS, 20 mM Hepes pH 7.3 added. Supernatants were harvested 16 hours after IL-1 induction and assayed for stromelysin expression by ELISA. Polyclonal antibody against Matrix Metalloproteinase 3 (Biogenesis, NH) was used as the detecting antibody and anti-stromelysin monoclonal antibody was used as the capturing antibody in the sandwich ELISA (Maniatis et al., *supra*) to measure stromelysin expression.

Example 12: Ribozyme-Mediated Inhibition of Stromelysin Expression in human fibroblast cells

Referring to Figs. 8 through 13, HH ribozymes, targeted to sites 21, 463, 1049, 1366, 1403, 1410 and 1489 within human stromelysin-1 mRNA, were transfected into HS-27 fibroblast or HSF cell line as described above. Catalytically inactive ribozymes that contain 2 nucleotide changes in the catalytic core region were also synthesized for use as controls. The catalytic core in the inactive ribozymes was CUUAUGAGGCCGAAAGGCCGAU versus CUGAUGAGGCCGAAAGGCCGAA in the active ribozymes. The inactive ribozymes show no cleavage activity *in vitro* when measured on full-length RNA in the typical ribozyme cleavage assay at a 1 μM concentration for 1 hour. Levels of stromelysin protein were measured using a sensitive ELISA protocol as described above. + IL-1 in the figures mean that cells were treated with IL-1 to induce the expression of stromelysin expression. -IL-1 means that the cells were not treated. Figs. 8 through 13 show the dramatic reduction in the levels of stromelysin protein expressed in cells that were transfected with active HH ribozymes. This decrease in the level of

stromelysin production is over and above some non-specific inhibition seen in cells that were transfected with catalytically inactive ribozymes. There is on an average a greater than 50% inhibition in stromelysin production (in cells transfected with active HH ribozymes) when compared with control cells that were transfected with inactive ribozymes. These results suggest that the reduction in stromelysin production in HS-27 cells is mediated by sequence-specific cleavage of human stromelysin-1 mRNA by catalytically active HH ribozymes. Reduction in stromelysin protein production in cells transfected with catalytically inactive ribozymes may be due to some "antisense effect" caused by binding of the inactive ribozyme to the target RNA and physically preventing translation.

Example 13: Ribozyme-mediated inhibition of stromelysin expression in Rabbit Knee

In order to extend the ribozyme efficacy in cell culture, applicant has chosen to use rabbit knee as a reasonable animal model to study ribozyme-mediated inhibition of rabbit stromelysin protein expression. Applicant selected a HH ribozyme (1049HH), targeted to site 1049 within human stromelysin-1 mRNA, for animal studies because site 1049 is 100% identical to site 1060 (Tables AIII and AVI) within rabbit stromelysin mRNA. This has enabled applicant to compare the efficacy of the same ribozyme in human as well as in rabbit systems.

Male New Zealand White Rabbits (3-4 Kg) were anaesthetized with ketamine-HCl/xylazine and injected intra-articularly (I.T.) in both knees with 100 µg ribozyme (e.g., SEQ. ID. NO. 202) in 0.5 ml phosphate buffered saline (PBS) or PBS alone (Controls). The IL-1 (human recombinant IL-1 α , 25 ng) was administered I.T., 24 hours following the ribozyme administration. Each rabbit received IL-1 in one knee and PBS alone in the other. The synovium was harvested 6 hours post IL-1 infusion, snap frozen in liquid nitrogen, and stored at -80°C. Total RNA is extracted with TRIzol reagent (GIBCO BRL, Gaithersburg, MD), and was analyzed by Northern-blot analysis and/or RNase-protection assay. Briefly, 0.5 µg cellular RNA was separated on 1.0 % agarose/formaldehyde gel and transferred to Zeta-Probe GT nylon membrane (Bio-Rad, Hercules, CA) by capillary transfer for ~16 hours. The blots were

baked for two hours and then pre-hybridized for 2 hours at 65°C in 10 ml Church hybridization buffer (7 % SDS, 500 mM phosphate, 1 mM EDTA, 1% Bovine Serum Albumin). The blots were hybridized at 65°C for ~16 hours with 10⁶ cpm/ml of full length ³²P-labeled complementary RNA (cRNA) probes to rabbit stromelysin mRNA (cRNA added to the pre-hybridization buffer along with 100 µl 10mg/ml salmon sperm DNA). The blot was rinsed once with 5% SDS, 25 mM phosphate, 1 mM EDTA and 0.5% BSA for 10 min at room temperature. This was followed by two washes (10 min each wash) with the same buffer at 65°C, which was then followed by two washes (10 min each wash) at 65°C with 1% SDS, 25 mM phosphate and 1 mM EDTA. The blot was autoradiographed. The blot was reprobbed with a 100 nt cRNA probe to 18S rRNA as described above. Following autoradiography, the stromelysin expression was quantified on a scanning densitometer, which is followed by normalization of the data to the 18S rRNA band intensities.

As shown in Figs. 14-16, catalytically active 1049HH ribozyme mediates a decrease in the expression of stromelysin expression in rabbit knees. The inhibition appears to be sequence-specific and ranges from 50-70%.

Example 14: Phosphorothioate-substituted Ribozymes inhibit stromelysin expression in Rabbit Knee

Ribozymes containing four phosphorothioate linkages at the 5' termini enhance ribozyme efficacy in mammalian cells. Referring to Fig. 17, applicant has designed and synthesized hammerhead ribozymes targeted to site 1049 within stromelysin RNA, wherein, the ribozymes contain five phosphorothioate linkages at their 5' and 3' termini. Additionally, these ribozymes contain 2'-O-methyl substitutions at 30 nucleotide positions, 2'-C-allyl substitution at U4 position and 2'-OH at five positions (Fig 17A). As described above, these ribozymes were administered to rabbit knees to test for ribozyme efficacy. The 1049 U4-C-allyl P=S active ribozyme shows greater than 50 % reduction in the level of stromelysin RNA in rabbit knee. Catalytically inactive version of the 1049 U4-C-allyl P=S ribozyme shows ~30% reduction in the level of stromelysin RNA.

Referring to Fig. 18, applicant has also designed and synthesized hammerhead ribozymes targeted to three distinct sites within stromelysin RNA, wherein, the ribozymes contain four phosphorothioate linkages at their 5' termini. Additionally, these ribozymes contain 2'-O-methyl substitutions at 29
5 nucleotide positions, 2'-amino substitutions at U4 and U7 positions and 2'-OH at five positions. As described above, these ribozymes were administered to rabbit knees to test for ribozyme efficacy. As shown in Figures 18-21, ribozymes targeted to sites 1049, 1363 and 1366 are all efficacious in rabbit knee. All three ribozymes decreased the level of stromelysin RNA in rabbit
10 knee by about 50 %.

Sequences and chemical modifications described in figures 17 and 18 are meant to be non-limiting examples. Those skilled in the art will recognize that similar embodiments with other ribozymes and ribozymes containing other chemical modifications can be readily generated using techniques
15 known in the art and are within the scope of the present invention.

Applicant has shown that chemical modifications, such as 6-methyl U and abasic (nucleotide containing no base) moieties can be substituted at certain positions within the ribozyme, for example U4 and U7 positions, without significantly effecting the catalytic activity of the ribozyme. Similarly,
20 3'-3' linked abasic inverted ribose moieties can be used to protect the 3' ends of ribozymes in place of an inverted T without effecting the activity of the ribozyme.

B7-1, B7-2, B7-3 and CD40 are attractive ribozyme targets by several criteria. The molecular mechanism of T cell activation is well-established.
25 Efficacy can be tested in well-defined and predictive animal models. The clinical end-point of graft rejection is clear. Since delivery would be *ex vivo*, treatment of the correct cell population would be assured. Finally, the disease condition is serious and current therapies are inadequate. Whereas protein-based therapies would induce anergy against all antigens encountered during
30 the several week treatment period, *ex vivo* ribozyme therapy provides a direct and elegant approach to truly donor-specific anergy.

Similarly, autoimmune diseases and allergies can be prevented or treated by reversing the devastating course of immune response to self-antigens. Specifically, nucleic acids of this inventions can dampen the response to naturally occurring antigens.

5 Example 15: B7-1, B7-2, B7-3 and/or CD40 Hammerhead ribozymes

By engineering ribozyme motifs we have designed several ribozymes directed against B7-1, B7-2, B7-3 and/or CD40 encoded mRNA sequences. These ribozymes were synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave target sequences *in*
10 *vitro* was evaluated.

Several common human cell lines are available that can be induced to express endogenous B7-1, B7-2, B7-3 and/or CD40 . Alternatively, murine splenic cells can be isolated and induced, to express B7-1 or B7-2, with IL-4 or recombinant CD40 ligand. B7-1 and B7-2 can be detected easily with
15 monoclonal antibodies. Use of appropriate fluorescent reagents and fluorescence-activated cell-sorting (FACS) will permit direct quantitation of surface B7-1 and B7-2 on a cell-by-cell basis. Active ribozymes are expected to directly reduce B7-1 or B7-2 expression. Ribozymes targeted to CD40 would prevent induction of B7-2 by CD40 ligand.

20 Several animal models of transplantation are available – Mouse, rat, Porcine model (Fodor et al., 1994, *Proc. Natl. Acad. Sci. USA* 91, 11153); or Baboon (reviewed by Nowak, 1994 *Science* 266, 1148). B7-1, B7-2, B7-3 and/or CD40 protein levels can be measured clinically or experimentally by FACS analysis. B7-1, B7-2, B7-3 and/or CD40 encoded mRNA levels will be
25 assessed by Northern analysis, RNase-protection, primer extension analysis and/or quantitative RT-PCR. Ribozymes that block the induction of B7-1, B7-2, B7-3 and/or CD40 activity and/or B7-1, B7-2, B7-3 and/or CD40 protein encoding mRNAs by more than 20% *in vitro* will be identified.

Several animals models of autoimmune disorders are available– allergic
30 encephalomyelitis (EAE) in Lewis rats (Carlson et al., 1993 *Ann. N.Y. Acad. Sci.* 685, 86); animal models of multiple sclerosis (Wekerle et al., 1994 *Ann.*

Neurol. 36, s47) and rheumatoid arthritis (van Laar et al., 1994 Chem. Immunol. 58, 206).

Several animal models of allergy are available and are reviewed by Kemeny and Diaz-Sanchez, 1990, Clin. Exp. Immunol. 82, 423 and Pretolani et al., 1994 Ann. N.Y.Acad. Sci. 725, 247).

RNA ribozymes and/or genes encoding them will be delivered by either free delivery, liposome delivery, cationic lipid delivery, adeno-associated virus vector delivery, adenovirus vector delivery, retrovirus vector delivery or plasmid vector delivery in these animal model experiments (see above). One dose of a ribozyme vector that constitutively expresses the ribozyme or one or more doses of a stable anti-B7-1, B7-2, B7-3 and/or CD40 ribozymes or a transiently expressing ribozyme vector to donor APC, followed by infusion into the recipient may reduce the incidence of graft rejection. Alternatively, graft tissues may be treated as described above prior to transplantation.

Example 16: Synthesis of 6-methyl-uridine phosphoramidite

Referring to Figure 30, the suspension of 6-methyl-uracil (2.77g, 21.96 mmol) in the mixture of hexamethyldisilazane (50mL) and dry pyridine (50mL) was refluxed for three hours. The resulting clear solution of trimethylsilyl derivative of 6-methyl uracyl was evaporated to dryness and coevaporated 2 times with dry toluene to remove traces of pyridine. To the solution of the resulting clear oil, in dry acetonitrile, 1-O-acetyl-2',3',5'-tri-O-benzoyl-b-D-ribose (10.1g, 20 mmol) was added and the reaction mixture was cooled to 0°C. To the above stirred solution, trimethylsilyl trifluoromethanesulfonate (4.35 mL, 24 mmol) was added dropwise and the reaction mixture was stirred for 1.5 h at 0°C and then 1h at room temperature. After that the reaction mixture was diluted with dichloromethane washed with saturated sodium bicarbonate and brine. The organic layer was evaporated and the residue was purified by flash chromatography on silica gel with ethylacetate-hexane (2:1) mixture as an eluent to give 9.5g (83%) of the compound 2 and 0.8g of the corresponding N¹,N³-bis-derivative.

To the cooled (-10°C) solution of the compound (4.2g, 7.36 mmol) in the mixture of pyridine (60 mL) and methanol (10 mL) ice-cooled 2M aqueous

5 solution of sodium hydroxide (16 mL) was added with constant stirring. The reaction mixture was stirred at -10°C for additional 30 minutes and then neutralized to pH 7 with Dowex 50 (Py⁺). The resin was filtered off and washed with a 200 mL mixture of H₂O - Pyridine (4:1). The combined "mother liquor" and the washings were evaporated to dryness and dried by multiple coevaporation with dry pyridine. The residue was redissolved in dry pyridine and then mixed with dimethoxytrityl chloride (2.99g, 8.03 mmol). The reaction mixture was left overnight at room temperature. Reaction was quenched with methanol (25 mL) and the mixture was evaporated. The residue was
10 dissolved in dichloromethane, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel using linear gradient of MeOH (2% to 5%) in CH₂Cl₂ as eluent to give 3.4g (83%) of the compound 6.

15 Example 17: Synthesis of 6-methyl-cytidine phosphoramidite

Triethylamine (13.4 ml, 100 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (6.22g, 90 mmol) and phosphorous oxychloride (1.89 ml, 20 mmol) in 50 ml of anhydrous acetonitrile. To the resulting suspension the solution of 2',3',5'-tri-O-Benzoyl-6-methyl uridine
20 (5.7g, 10 mmol) in 30 ml of acetonitrile was added dropwise and the reaction mixture was stirred for 4 hours at room temperature. Then it was concentrated in vacuo to minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was
25 removed in vacuo. The residue was dissolved in 100 ml of 1,4-dioxane and treated with 50 mL of 29% aq NH₄OH overnight. The solvents were removed in vacuo. The residue was dissolved in the mixture of pyridine (60 mL) and methanol (10 mL), cooled to -15°C and ice-cooled 2M aq solution of sodium hydroxide was added under stirring. The reaction mixture was stirred
30 at -10 to -15°C for additional 30 minutes and then neutralized to pH 7 with Dowex 50 (Py⁺). The resin was filtered off and washed with 200 mL of the mixture H₂O - Py (4:1). The combined mother liquor and washings were evaporated to dryness. The residue was crystallized from aq methanol to give 1.6g (62%) of 6-methyl cytidine.

To the solution of 6-methyl cytidine (1.4g, 5.44 mmol) in dry pyridine 3.11 mL of trimethylchlorosilane was added and the reaction mixture was stirred for 2 hours at room temperature. Then acetic anhydride (0.51 mL, 5.44 mmol) was added and the reaction mixture was stirred for additional 3 hours at room temperature. TLC showed disappearance of the starting material and the reaction was quenched with MeOH (20 mL), ice-cooled and treated with water (20 mL, 1 hour). The solvents were removed in vacuo and the residue was dried by four coevaporations with dry pyridine. Finally it was redissolved in dry pyridine and dimethoxytrityl chloride (2.2 g, 6.52 mmol) was added. The reaction mixture was stirred overnight at room temperature and quenched with MeOH (20 mL). The solvents were removed in vacuo. The remaining oil was dissolved in methylene chloride, washed with saturated sodium bicarbonate and brine. The organic layer was separated and evaporated and the residue was purified by flash chromatography on silica gel with the gradient of MeOH in methylene chloride (3% to 5%) to give 2.4 g (74%) of the compound (4).

Example 18: Synthesis of 6-aza-uridine and 6-aza-cytidine

To the solution of 6-aza uridine (5g, 20.39 mmol) in dry pyridine dimethoxytrityl chloride (8.29g, 24.47 mmol) was added and the reaction mixture was left overnight at room temperature. Then it was quenched with methanol (50 mL) and the solvents were removed in vacuo. The remaining oil was dissolved in methylene chloride and washed with saturated aq sodium bicarbonate and brine. The organic layer was separated and evaporated to dryness. The residue was additionally dried by multiple coevaporations with dry pyridine and finally dissolved in dry pyridine. Acetic anhydride (4.43 mL, 46.7 mmol) was added to the above solution and the reaction mixture was left for 3 hours at room temperature. Then it was quenched with methanol and worked-up as above. The residue was purified by flash chromatography on silica gel using mixture of 2% of MeOH in methylene chloride as an eluent to give 9.6g (75%) of the compound.

Triethylamine (23.7 ml, 170.4 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (10.6g, 153.36 mmol) and phosphorous oxychloride (3.22 ml, 34.08 mmol) in 100 ml of anhydrous acetonitrile. To the resulting suspension the solution of 2',3'-di-O-Acetyl-5'-O-Dimethoxytrityl-6-

aza Uridine (7.13g, 11.36 mmol) in 40 ml of acetonitrile was added dropwise and the reaction mixture was stirred for 6 hours at room temperature. Then it was concentrated in vacuo to minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed in vacuo. The residue was dissolved in 150 ml of 1,4-dioxane and treated with 50 mL of 29% aq NH_4OH for 20 hours at room temperature. The solvents were removed in vacuo. The residue was purified by flash chromatography on silica gel using linear gradient of MeOH (4% to 10%) in methylene chloride as an eluent to give 3.1g (50%) of azacytidine.

To the stirred solution of 5'-O-Dimethoxytrityl-6-aza cytidine (3g, 5.53 mmol) in anhydrous pyridine trimethylchloro silane (2.41 mL, 19 mmol) was added and the reaction mixture was left for 4 hours at room temperature. Then acetic anhydride (0.63 mL, 6.64 mmol) was added and the reaction mixture was stirred for additional 3 hours at room temperature. After that it was quenched with MeOH (15 mL) and the solvents were removed in vacuo. The residue was treated with 1M solution of tetrabutylammonium fluoride in THF (20°, 30 min) and evaporated to dryness.. The remaining oil was dissolved in methylene chloride, washed with saturated aq sodium bicarbonate and water. The separated organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica gel using 4% MeOH in methylene chloride as an eluent to give 2.9g (89.8%) of the compound.

General Procedure for the Introducing of the TBDMS-Group: To the stirred solution of the protected nucleoside in 50 mL of dry THF and pyridine (4 eq) AgNO_3 (2.4 eq) was added. After 10 minutes tert-butyldimethylsilyl chloride (1.5 eq) was added and the reaction mixture was stirred at room temperature for 12 hours. The resulted suspension was filtered into 100 mL of 5% aq NaHCO_3 . The solution was extracted with dichloromethane (2x100 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography on silica gel with hexanes-ethylacetate (3:2) mixture as eluent.

General Procedure for Phosphitylation: To the ice-cooled stirred solution of protected nucleoside (1 mmol) in dry dichloromethane (20 mL) under argon blanket was added dropwise via syringe the premixed solution of N,N-diisopropylethylamine (2.5eq) and 2-cyanoethyl N'N-diisopropylchlorophosphoramidite (1.2 eq) in dichloromethane (3 mL). Simultaneously via another syringe N-methylimidazole (1 eq) was added and stirring was continued for 2 hours at room temperature. After that the reaction mixture was again ice-cooled and quenched with 15 ml of dry methanol. After 5 min stirring, the mixture was concentrated in vacuo (<40°C) and purified by flash chromatography on silica gel using hexanes-ethylacetate mixture contained 1% triethylamine as an eluent to give corresponding phosphoroamidite as white foam.

Example 19: RNA cleavage activity of HHA ribozyme substituted with 6-methyl-Uridine

Hammerhead ribozymes targeted to site A (see Fig. 31) were synthesized using solid-phase synthesis, as described above. U4 position was modified with 6-methyl-uridine.

RNA cleavage assay *in vitro*:

Substrate RNA is 5' end-labeled using [γ -³²P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂. The reaction is initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gel. The results are quantified and percentage of target RNA cleaved is plotted as function of time.

Referring to Fig. 32, hammerhead ribozymes containing 6-methyl-uridine modification at U4 position cleave the target RNA efficiently.

Example 20: RNA cleavage activity of HHB ribozyme substituted with 6-methyl-Uridine

5 Hammerhead ribozymes targeted to site B (see Fig. 33) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were modified with 6-methyl-uridine.

RNA cleavage reactions were carried out as described above. Referring to Fig. 34, hammerhead ribozymes containing 6-methyl-uridine modification at U4 and U7 positions cleave the target RNA efficiently.

10 Example 21: RNA cleavage activity of HHC ribozyme substituted with 6-methyl-Uridine

Hammerhead ribozymes targeted to site C (see Fig. 35) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were modified with 6-methyl-uridine.

15 RNA cleavage reactions were carried out as described above. Referring to Fig. 36, hammerhead ribozymes containing 6-methyl-uridine modification at U4 positions cleave the target RNA efficiently.

20 Sequences listed in Figure 23, 31, 33, 35, and others and the modifications described in these figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

25 Example 22: Inhibition of Rat smooth muscle cell proliferation by 6-methyl-U substituted ribozyme HHA.

Hammerhead ribozyme (HHA) is targeted to a unique site (site A) within *c-myb* mRNA. Expression of *c-myb* protein has been shown to be essential for the proliferation of rat smooth muscle cell (Brown *et al.*, 1992 *J. Biol. Chem.* 267, 4625).

The ribozymes that cleaved site A within *c-myb* RNA described above were assayed for their effect on smooth muscle cell proliferation. Rat vascular smooth muscle cells were isolated and cultured as described (Stinchcomb *et al.*, *supra*). HHA ribozymes were complexed with lipids and delivered into rat smooth muscle
5 cells. Serum-starved cells were stimulated as described by Stinchcomb *et al.*, *supra*. Briefly, serum-starved smooth muscle cells were washed twice with PBS, and the RNA/lipid complex was added. The plates were incubated for 4 hours at 37°C. The medium was then removed and DMEM containing 10% FBS, additives and 10 µM bromodeoxyuridine (BrdU) was added. In some wells, FBS was
10 omitted to determine the baseline of unstimulated proliferation. The plates were incubated at 37°C for 20-24 hours, fixed with 0.3% H₂O₂ in 100% methanol, and stained for BrdU incorporation by standard methods. In this procedure, cells that have proliferated and incorporated BrdU stain brown; non-proliferating cells are counter-stained a light purple. Both BrdU positive and BrdU negative cells were
15 counted under the microscope. 300-600 total cells per well were counted. In the following experiments, the percentage of the total cells that have incorporated BrdU (% cell proliferation) is presented. Errors represent the range of duplicate wells. Percent inhibition then is calculated from the % cell proliferation values as follows: % inhibition = 100 - 100 (Ribozyme - 0% serum)/(Control - 0% serum).

20 Referring to Figure 37, active ribozymes substituted with 6-methyl-U at position 4 of HHA were successful in inhibiting rat smooth muscle cell proliferation. A catalytically inactive ribozyme (inactive HHA), which has two base substitutions within the core (these mutations inactivate a hammerhead ribozyme; Stinchcomb *et al.*, *supra*), does not significantly inhibit rat smooth muscle cell proliferation.

25 Example 23: Inhibition of stromelysin production in human synovial fibroblast cells by 6-methyl-U substituted ribozyme HHC.

Hammerhead ribozyme (HHC) is targeted to a unique site (site C) within *stromelysin* mRNA.

30 The general assay was as described (Draper *et al.*, *supra*). Briefly, fibroblasts, which produce stromelysin, are serum-starved overnight and ribozymes or controls are offered to the cells the next day. Cells were maintained in serum-free media. The ribozyme were applied to the cells as free ribozyme, or in association with various delivery vehicles such as cationic

lipids (including Transfectam™, Lipofectin™ and Lipofectamine™), conventional liposomes, non-phospholipid liposomes or biodegradable polymers. At the time of ribozyme addition, or up to 3 hours later, Interleukin-1 α (typically 20 units/ml) can be added to the cells to induce a large increase in stromelysin expression. The production of stromelysin can then be monitored over a time course, usually up to 24 hours.

Supernatants were harvested 16 hours after IL-1 induction and assayed for stromelysin expression by ELISA. Polyclonal antibody against Matrix Metalloproteinase 3 (Biogenesis, NH) was used as the detecting antibody and anti-stromelysin monoclonal antibody was used as the capturing antibody in the sandwich ELISA (Maniatis *et al.*, *supra*) to measure stromelysin expression.

Referring to Figure 38, HHC ribozyme containing 6-methyl-U modification, caused a significant reduction in the level of stromelysin protein production. Catalytically inactive HHC had no significant effect on the protein level.

Example 24: Synthesis of pyridin-2(4)-one nucleoside 3'-phosphoramidites

General procedure for the preparation of 1-(2,3,5-tri-O-benzoyl- β -D-ribofuranosyl)-2(4)-pyridones (3) and (9)

Referring to Figure 39, 2- or 4-hydroxypyridine (1) or (8) (2.09 g, 22 mmol), 1-O-acetyl-2,3,5-tri-O-benzoyl- β -D-ribofuranose (2) (10.08 g, 20 mmol) and BSA (5.5 ml, 22 mmol) were dissolved in dry acetonitrile (100 ml) under argon at 70°C (oil bath) and the mixture stirred for 10 min. Trimethylsilyl trifluoromethanesulfonate (TMSTf) (5.5 ml, 28.5 mmol) was added and the mixture was stirred for an additional hour for 1 or four hours for 8. The mixture was then cooled to room temperature (RT) followed by dilution, with CHCl₃ (200 ml), and extraction, with sat. aq. NaHCO₃ solution. The organic layer was washed with brine, dried (Na₂SO₄) and evaporated to dryness *in vacuo*. The residue was chromatographed on the column of silica gel; 1-5% gradient of methanol in dichloromethane was used for purification of 3 (98% yield) and 2-10% gradient of methanol in dichloromethane for purification of 9 (84% yield).

1-(β -D-Ribofuranosyl)-2(4)-pyridones (4) and (10)

3 or 9 (18 mmol) was dissolved in 0.3M NaOCH₃ (150 ml) and the solution was stirred at RT for 1 hour. The mixture was then neutralized, with Dowex 50WX8 (Py⁺), the ion-exchanger was filtered off and the filtrate was concentrated to a syrup *in vacuo*. The residue was dissolved in water (100 ml) and the solution was washed with chloroform (2 x 50 ml) and ether (2 x 50 ml). The aqueous layer was evaporated to dryness and the residue was then crystallized from ethyl acetate (3.9 g, 91% 4; Niedballa *et al.*, *Nucleic Acid Chemistry*, Part 1, Townsend, L.B. and Tipson, R.S., Ed.; J. Wiley & Sons, Inc.; New York, 1978, p 481-484); 10 (Niedballa and Vorbrüggen, *J. Org. Chem.* 1974, 39, 3668-3671) was crystallized from ethanol (3.6 g, 84%).

1-(2-O-TBDMSi-5-O-DMT- β -D-ribofuranosyl)-2(4)-pyridones

4 or 10 was 5'-O-dimethoxytritylated according to the standard procedure (see *Oligonucleotide Synthesis: A Practical Approach*, M.J. Gait Ed.; IRL Press, Oxford, 1984, p 27) to yield 5 in 76% yield and pyridin-4-one derivative in 67% yield in the form of yellowish foams after silica gel column chromatography (0.5-10% gradient of methanol in dichloromethane). These compounds were treated with *t*-butyldimethylsilyl chloride under the conditions described by Hakimelahi *et al.*, *Can. J. Chem.* 1982, 60, 1106-1113, and the reaction mixtures were purified by the silica gel column chromatography (20-50% gradient of ethyl acetate in hexanes) to enable faster moving 2'-O-TBDMSi isomers (68.5% and 55%, respectively) as colorless foams.

1-[2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)]-2(4)-pyridones (7) and (11)

1-(2-O-TBDMS-5-O-DMT- β -D-ribofuranosyl)-2(4)-pyridones were phosphorylated under conditions described by Tuschl *et al.*, *Biochemistry* 1993, 32, 11658-11668, and the products were isolated by silica gel column chromatography using 15-50% gradient of ethyl acetate in hexanes (1% Et₃N) for 7 (89% yield) and dichloromethane (1% Et₃N) for 11 (94% yield).

Phosphoramidites 7 and 11 were incorporated into ribozymes and substrates using the method of synthesis, deprotection, purification and testing

previously described (Wincott *et al.*, 1995 *supra*). The average stepwise coupling yields were ~98 %.

Example 25: Synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-phenyl- β -D-ribofuranose (8) phosphoramidites

5-*O*-*t*-Butyldiphenylsilyl-2,3-*O*-isopropylidene-1-deoxy-1-phenyl- β -D-ribofuranose (3)

Referring to Figure 40, compound 3 was prepared using the procedure analogous to that described by Czerniecki and Ville, *J. Org. Chem.* **1989**, *54*, 610-612. Contrary to their result, we succeeded in obtaining the title compound, by using the more acid resistant *t*-butyldiphenylsilyl group for 5-*O*-protection, instead of *t*-butyldimethylsilyl.

1-Deoxy-1-phenyl- β -D-ribofuranose (5)

Compound 3 (1 g, 2.05 mmol) was dissolved in THF (20 ml) and the solution was mixed with 1M TBAF in THF (3 ml, 3 mmol). The reaction mixture was stirred at RT for 30 min followed by evaporation into a syrup. The residue was applied on to a silica gel column and eluted with hexanes followed by 5-70% gradient of ethyl acetate in hexanes. The 5-*O*-desilylated product was obtained as a colorless foam (0.62 g, 88% yield). This material was dissolved in 70% acetic acid and heated at 100°C (oil bath) for 30 min. Evaporation to dryness under reduced pressure and crystallization of the residual syrup from toluene resulted in 5 (0.49 g, 94% yield), mp 120-121°C.

2-*O*-*t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-1-deoxy-1-phenyl- β -D-ribofuranose (7)

Compound 5 (770 mg, 3.66 mmol) was 5-*O*-dimethoxytritylated according to the standard procedure (Oligonucleotide Synthesis: A Practical Approach, M.J. Gait Ed.; IRL Press, Oxford, 1984, p 27) to yield 1.4 g (75% yield) of 5-*O*-dimethoxytrityl derivative as a yellowish foam, following silica gel column chromatography (0.5-2% gradient of methanol in dichloromethane). This material was treated with *t*-butyldimethylsilyl chloride under the conditions described by Hakimelahi *et al.*, *Can. J. Chem.* **1982**, *60*, 1106-1113, and the reaction mixture

was purified by silica gel column chromatography (2-10% gradient of ethyl acetate in hexanes) to afford a slower moving 2'-O-TBDMSi isomer 7 (0.6 g, 3% yield) as a colorless foam. The faster migrating 3'-O-TBDMSi isomer 6 was also isolated (0.55 g, 32% yield).

5 2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-phenyl- β -D-ribofuranose (8)

Compound 7 (0.87 g, 1.39 mmol) was phosphitylated under conditions described by Tuschl *et al.*, *supra* and the product was isolated by silica gel column chromatography using 0.5% ethyl acetate in toluene (1% Et₃N) for elution. (0.85 g, 74% yield).

10 Example 26: Synthesis of pseudouridine, 3-methyluridine and 2,4,6-trimethoxy benzene nucleoside phosphoramidites

Starting with a pseudo uridine, 3-methyluridine or 2,4,6-trimethoxy benzene nucleoside (Gasparutto *et al.*, *Nucleic Acid Res.* 1992 20, 5159-5166; Kalvo and Farkas, *Nucleic Acid Chemistry*, Part 1, Townsend, L.B. and Tipson, R.S., E. J. Wiley & Sons, Inc.; New York, 1978, p 481-484), phosphoramidites can be prepared by standard protocols described below (Figure 41).

General Procedure for the Introducing of the TBDMS-Group: To the stirred solution of the protected nucleoside in 50 mL of dry THF and pyridine (4 eq) AgNO₃ (2.4 eq) was added. After 10 minutes tert-butyldimethylsilyl chloride (1 eq) was added and the reaction mixture was stirred at room temperature for 24 hours. The resulted suspension was filtered into 100 mL of 5% aq NaHCO₃. The solution was extracted with dichloromethane (2x100 mL). The combined organic layer was washed with brine, dried over Na₂SO₄ and evaporated. The residue was purified by flash chromatography on silica gel with hexanes-ethylacetate (3:1 mixture as eluent).

General Procedure for Phosphitylation: To the ice-cooled stirred solution of protected nucleoside (1 mmol) in dry dichloromethane (20 mL) under argon blanket was added dropwise via syringe the premixed solution of *N,N*-diisopropylethylamine (2.5eq) and 2-cyanoethyl *N*'-(diisopropylchlorophosphoramidite) (1.2 eq) in dichloromethane (3 mL).

Simultaneously via another syringe N-methylimidazole (1 eq) was added and stirring was continued for 2 hours at room temperature. After that the reaction mixture was again ice-cooled and quenched with 15 ml of dry methanol. After 5 min stirring, the mixture was concentrated in vacuo (<40°C) and purified by flash chromatography on silica gel using hexanes-ethylacetate mixture contained 1% triethylamine as an eluent to give corresponding phosphoroamidite as white foam.

Pseudouridine, 3-methyluridine or 2,4,6-trimethoxy benzene phosphoramidites were incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. The ribozymes were deprotected using the standard protocol described above with the exception of ribozymes with pseudouridine. Pseudouridine-modified ribozymes were deprotected first by incubation at room temperature, instead of at 55°C, for 24 hours in a mixture of ethanolic ammonia (3:1).

15 Example 27: Synthesis of dihydrouridine phosphoramidites

Referring to Figure 42, dihydrouridine phosphoramidite was synthesized based on the method described in Chaix *et al.*, 1989 *Nucleic Acid Res.* 17, 7381-7393 with certain improvements:

20 i. Uridine (1; 10g, 41mmoles) was dissolved in 200 ml distilled water and to the solution 2g of Rh (10% on alumina) was added. The slurry was brought to 60 psi of hydrogen, and hydrogenation was continued for 16hrs. Reaction was monitored by disappearance of UV absorbing material. All of starting material was converted to dihydrouridine (DHU) and tetrahydrouridine (2:1 based on NMR). Tetrahydrouridine was not removed at this step.

25 ii. Dihydrouridine (2; 10g, 41mmoles) was dissolved in 400ml dry pyridine; dimethylaminopyridine (0.244g, 2mmoles), triethylamine (7.93ml, 56mmoles), and dimethoxytritylchloride (16.3g, 48mmoles) were added and stirred under argon overnight. The reaction was quenched with 50ml methanol, extracted with 400ml 5% sodium bicarbonate, and then 400ml brine. The organic phase was dried over sodium sulphate, filtered, and then dried to a foam. 5'-DMT-DHU (3) was purified by silica gel chromatography (dichloromethane with 0.5-5% gradient of methanol; final yield = 9g; 16.4mmoles).

iii. 5'-DMT-DHU (3; 9.0g, 16.4mmoles) was dissolved in 150ml dry THF. Pyridine (4.9ml, 60mmoles) and silver nitrate (3.35g, 19.7mmoles) were added at room temperature and stirred under argon for 10min., then tert.-butyldimethylsilylchloride (tBDMS-Cl; 3.0g, 19.7mmoles) was added and the slurry
5 was stirred under argon overnight. The reaction was filtered over celite into 500ml aqueous 5% sodium bicarbonate and then extracted with 200ml chloroform. The organic phase was washed with 250ml brine, dried over sodium sulfate, and then evaporated to a yellow foam. 2'-tBDMS, 5'-DMT-DHU (5) was purified by silica gel chromatography away from the 3'-tBDMS, 5'-DMT-DHU (4) (hexanes with 10-50%
10 gradient ether; final yield = 5.1g; 7.7mmoles), dried over sodium sulfate, filtered, and then dried to a white powder. The product was kept under high vacuum for 48hrs.

iv. 5'-DMT, 2'-tBDMS-DHU (5; 2.10g, 3.17mmoles) was dissolved in 40ml anhydrous dichloromethane. NN-dimethylaminopyridine (2.21ml, 12.7mmoles), N-methylimidazole (1.27ml, 1.59mmoles), and chloro-diisopropyl-
15 cyanoethylphosphoramidite (1.2ml, 5.22mmoles) were added and the reaction was stirred under argon for 3hrs. The reaction was quenched with 4ml anhydrous methanol and then evaporated to an oil. Final product (6) was purified by silica gel chromatography (dichloromethane with 0-1% ethanol; 1% triethylamine; final
20 yield = 2.2g; 2.5mmoles).

The dihydrouridine was incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. with improvements—nucleoside-oxalyl-polystyrene derivatized support (Alul *et. al.* Nucleic Acids Res., 1991, 19, 1527-1532) was used. The ribozyme containing the dihydrouridine
25 substitution was deprotected using 30% methyl amine in anhydrous ethanol for 15 min. at room temperature and subsequent treatment with *tert*-butyl-ammonium fluoride in anhydrous THF for 24 hrs. at room temperature.

Example 28: Synthesis of 2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-1-deoxy-1-naphthyl- β -D-ribofuranose (7) phosphoramidites
30

1-Deoxy-1-naphthyl- β -D-ribofuranose (4)

Referring to Figure 45, the title compound was synthesized from naphthalene 1 and tetra-*O*-acetyl- β -D-ribofuranose 2 according to the procedure of Ohrui *et al.* *Agr. Biol. Chem.* 1972, 36, 1651-1653.

5 2-*O*-*t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-naphthyl- β -D-ribofuranose (7)

7 was synthesized in three steps from 4: a) 5'-*O*-dimethoxytritylation using 4,4'-dimethoxytrityl triflate, followed by chromatographic separation of α and β anomer, respectively; b) 2'-*O*-silylation was carried out as described by Hakimelahi *et al.*, 1982 *supra* (32% yield); c) 3'-*O*-phosphitylation was carried out essentially as described by Tuschl *et al.*, 1993 *supra* (85% yield).

This phosphoramidite is incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. The ribozyme containing naphthyl substitution was deprotected using the standard protocol described above.

15 Example 29: Synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-Deoxy-1-(*p*-Aminophenyl)- β -D-Ribofuranose phosphoramidites

5-*O*-*t*-Butyldiphenylsilyl-2,3-*O*-isopropylidene-1-deoxy-1-(*p*-bromophenyl)- β -D-ribofuranose (3)

20 Referring to Figure 46, 3 was prepared from 4-bromo-1-lithiobenzene and *t*-butyldiphenylsilyl-2,3-*O*-isopropylidene-D-ribo-1,4-lactone using the procedure analogous to that described by Czerniecki and Ville, *J. Org. Chem.* 1989, 54, 610-612. Contrary to their result, we succeeded in obtaining the title compound, by using instead of *t*-butyldimethylsilyl the more acid resistant
25 *t*-butyldiphenylsilyl group for 5-*O*-protection.

5-*O*-*t*-Butyldiphenylsilyl-2,3-*O*-isopropylidene-1-deoxy-1-(*p*-aminophenyl)- β -D-ribofuranose (5)

Compound 3 was aminated using liquid ammonia and CuI as described by Piccirilli *et al.* *Helv. Chim. Acta* 1991, 74, 397-406 to give the title
30 compound in 63% yield.

5-O-*t*-Butyldiphenylsilyl-2,3-O-isopropylidene-1-deoxy-1-[p-(N-TFA)aminophenyl]- β -D-ribofuranose (6)

5 (1.2 g, 2.88 mmol) in dry pyridine (20 ml) was treated with trifluoroacetic anhydride (0.5 ml, 3.6 mmol) for 1 hour at 0 °C. The reaction mixture was then quenched with methanol (5 ml) and evaporated to a syrup. The syrup was partitioned between 5% aq. NaHCO₃ and dichloromethane, organic layer was dried (Na₂SO₄) and evaporated to dryness under reduced pressure. This material was used without further purification in the next step.

1-Deoxy-1-[p-(N-TFA)aminophenyl]- β -D-ribofuranose (7)

10 The title compound was prepared from 6 in an identical manner as for the synthesis of deblocked phenyl analog; (82% overall yield for 5'-O-desilylation and the cleavage of 2',3'-O-isopropylidene group).

15 2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-[p-(N-TFA)aminophenyl]- β -D-ribofuranose (10)

Using the same three step sequence as for the phenyl analog, 10 was prepared from 7 in 32% overall yield.

20 This phosphoramidite is incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. The ribozyme containing aminophenyl substitution was deprotected using the standard protocol described above.

Example 30: RNA cleavage reactions catalyzed by HH-B substituted with modified bases

25 Hammerhead ribozymes targeted to site B (see Fig. 43A) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were substituted with various base-modifications shown in Figure 43B.

RNA cleavage reactions were carried out as described above. Referring to Fig. 43B, hammerhead ribozymes containing base modifications at positions 4 and 7 cleave the target RNA to varying degrees of efficiency. Some of the base modifications at position 7 appear to enhance the catalytic efficiency of the

30

hammerhead ribozymes compared to a standard base at that position (see Figure 43B, pyridin-4-one, phenyl and 3-methyl U modifications).

5 HH-B ribozymes with either pyridin-4-one or phenyl substitution at position 7 were further characterized (Figure 44). It appears that HH-B ribozyme with pyridin-4-one modification at position 7 cleaves RNA with a 10 fold higher k_{cat} when compared to a ribozyme with a U at position 7 (compare Figure 44 A with 44 B). HH-B ribozyme with a phenyl group at position 7 cleaves RNA with a 3 fold higher k_{cat} when compared to a hammerhead ribozyme with U at position 7 (see Figure 44C).

10 Sequences listed in Figure 23, 31, 33, 35, 43 and the modifications described in these figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides
15 and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 31: 2'-deoxy-2'-alkylnucleotides

20 Table D2 is a summary of specified catalytic parameters (t_A and t_S) on short substrates *in vitro*, and stabilities of the noted modified catalytic nucleic acids in human serum. U4 and U7 refer to the uracil bases noted in Figure 1. Modifications at the 2'-position are shown in the table.

Table D2

Entry	Modification	$t_{1/2}$ (m) Activity (t_A)	$t_{1/2}$ (m) Stability (t_S)	$\beta = t_S/t_A$ $\times 10$
1	U4 & U7 = U	1	0.1	1
2	U4 & U7 = 2'-O-Me-U	4	260	650
3	U4 = 2'=CH ₂ -U	6.5	120	180
4	U7 = 2'=CH ₂ -U	8	280	350
5	U4 & U7 = 2'=CH ₂ -U	9.5	120	130
6	U4 = 2'=CF ₂ -U	5	320	640
7	U7 = 2'=CF ₂ -U	4	220	550
8	U4 & U7 = 2'=CF ₂ -U	20	320	160
9	U4 = 2'-F-U	4	320	800
10	U7 = 2'-F-U	8	400	500
11	U4 & U7 = 2'-F-U	4	300	750
12	U4 = 2'-C-Allyl-U	3	>500	>1700
13	U7 = 2'-C-Allyl-U	3	220	730
14	U4 & U7 = 2'-C-Allyl-U	3	120	400
15	U4 = 2'-araF-U	5	>500	>1000
16	U7 = 2'-araF-U	4	350	875
17	U4 & U7 = 2'-araF-U	15	500	330
18	U4 = 2'-NH ₂ -U	10	500	500
19	U7 = 2'-NH ₂ -U	5	500	1000
20	U4 & U7 = 2'-NH ₂ -U	2	300	1500
21	U4 = dU	6	100	170
22	U4 & U7 = dU	4	240	600

Figure 47 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. Referring to Figure 47, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 2'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Although substitutions of any nucleotide with any of the modified nucleotides shown in Figure 48 are possible, and were indeed synthesized, the basic structure composed of primarily 2'-O-Me nucleotides with selected substitutions was chosen to maintain maximal catalytic activity (Yang *et al. Biochemistry* 1992, 31, 5005-5009 and Paoletta *et al. EMBO J.* 1992, 11, 1913-1919) and ease of synthesis, but is not limiting to this invention.

Ribozymes from Figure 47 and Table D2 were synthesized and assayed for catalytic activity and nuclease resistance. With the exception of entries 8 and 17, all of the modified ribozymes retained at least 1/10 of the wild-type catalytic activity. From Table D2, all 2'-modified ribozymes showed very large and significant increases in stability in human serum (shown) and in the other fluids described below (Example 3, data not shown). The order of most aggressive nuclease activity was fetal bovine serum > human serum > human plasma > human synovial fluid. As an overall measure of the effect of these 2'-substitutions on stability and activity, a ratio β was calculated (Table D2). This β value indicated that all modified ribozymes tested had significant, >100 - >1700 fold, increases in overall stability and activity. These increases in β indicate that the lifetime of these modified ribozymes *in vivo* are significantly increased which should lead to a more pronounced biological effect.

More general substitutions of the 2'-modified nucleotides from Figure 48 also increased the $t_{1/2}$ of the resulting modified ribozymes. However the catalytic activity of these ribozymes was decreased > 10-fold.

In Figure 53 compound 37 may be used as a general intermediate to prepare derivatized 2'-C-alkyl phosphoramidites, where X is CH₃, or an alkyl, or other group described above.

The following are other non-limiting examples showing the synthesis of nucleic acids using 2'-C-alkyl substituted phosphoramidites, the syntheses of the amidites, their testing for enzymatic activity and nuclease resistance. These examples are diagrammed in Figs 48-54.

5 Example 32: Synthesis of Hammerhead Ribozymes Containing 2'-Deoxy-2'-Alkyl nucleotides & Other 2'-Modified Nucleotides

10 The method of synthesis used generally follows the procedure for normal RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854 and in
15 Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* **1990**, *18*, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds **10**, **12**, **17**, **22**, **31**, **18**, **26**, **32**, **36** and **38**). Other 2'-modified phosphoramidites were prepared according to: **3** & **4**, Eckstein *et al.*
20 *International Publication No.* WO 92/07065; and **5** Kois *et al.* *Nucleosides & Nucleotides* **1993**, *12*, 1093-1109. The average stepwise coupling yields were ~98%. The 2'-substituted phosphoramidites were incorporated into hammerhead ribozymes as shown in Figure 5. However, these 2'-alkyl substituted phosphoramidites may be incorporated not only into hammerhead
25 ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 33: Ribozyme Activity Assay

25 Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 mM, 200 nM, 40 nM or 8 nM and the final substrate RNA concentrations were ~ 1 nM. Total reaction volumes were 50 mL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were initiated by mixing substrate and
30 ribozyme solutions at t = 0. Aliquots of 5 mL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each time point was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis.

Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

Example 34: Stability Assay

500 pmol of gel-purified 5'-end-labeled ribozymes were precipitated in ethanol and pelleted by centrifugation. Each pellet was resuspended in 20 mL of appropriate fluid (human serum, human plasma, human synovial fluid or fetal bovine serum) by vortexing for 20 s at room temperature. The samples were placed into a 37 °C incubator and 2 mL aliquots were withdrawn after incubation for 0, 15, 30, 45, 60, 120, 240 and 480 m. Aliquots were added to 20 mL of a solution containing 95% formamide and 0.5X TBE (50 mM Tris, 50 mM borate, 1 mM EDTA) to quench further nuclease activity and the samples were frozen until loading onto gels. Ribozymes were size-fractionated by electrophoresis in 20% acrylamide/8M urea gels. The amount of intact ribozyme at each time point was quantified by scanning the bands with a phosphorimager (Molecular Dynamics) and the half-life of each ribozyme in the fluids was determined by plotting the percent intact ribozyme vs the time of incubation and extrapolation from the graph.

Example 35: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-O-Phenoxythio-carbonyl-Uridine (7)

To a stirred solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-uridine, 6, (15.1 g, 31 mmol, synthesized according to *Nucleic Acid Chemistry*, ed. Leroy Townsend, 1986 pp. 229-231) and dimethylaminopyridine (7.57 g, 62 mmol) a solution of phenylchlorothionoformate (5.15 mL, 37.2 mmol) in 50 mL of acetonitrile was added dropwise and the reaction stirred for 8 h. TLC (EtOAc:hexanes / 1:1) showed disappearance of the starting material. The reaction mixture was evaporated, the residue dissolved in chloroform, washed with water and brine, the organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica gel with EtOAc:hexanes / 2:1 as eluent to give 16.44 g (85%) of 7.

Example 36: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl -Uridine (8)

To a refluxing, under argon, solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-O-phenoxythiocarbonyl-uridine, 7, (5 g, 8.03 mmol) and allyltributyltin (12.3 mL, 40.15 mmol) in dry toluene, benzoyl peroxide (0.5 g) was added portionwise during 1 h. The resulting mixture was allowed to reflux under argon for an additional 7-8 h. The reaction was then evaporated and the product 8 purified by flash chromatography on silica gel with EtOAc:hexanes / 1:3 as eluent. Yield 2.82 g (68.7%).

Example 37: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine (9)

A solution of 8 (1.25 g, 2.45 mmol) in 10 mL of dry tetrahydrofuran (THF) was treated with a 1 M solution of tetrabutylammoniumfluoride in THF (3.7 mL) for 10 m at room temperature. The resulting mixture was evaporated, the residue was loaded onto a silica gel column, washed with 1 L of chloroform, and the desired deprotected compound was eluted with chloroform:methanol / 9:1. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched with methanol (20 mL), evaporated, dissolved in chloroform, washed with 5% aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel, EtOAc:hexanes / 1:1 as eluent, to give 0.85 g (57%) of 9 as a white foam.

Example 38: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (10)

5'-O-Dimethoxytrityl-2'-C-allyl-uridine (0.64 g, 1.12 mmol) was dissolved in dry dichloromethane under dry argon. N,N-Diisopropylethylamine (0.39 mL, 2.24 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.35 mL, 1.57 mmol) was added dropwise to the stirred reaction solution and stirring was continued for 2 h at RT. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C)

and purified by flash chromatography on silica gel using a gradient of 10-60% EtOAc in hexanes containing 1% triethylamine mixture as eluent. Yield: 0.78 g (90%), white foam.

5 Example 39: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl-N⁴-Acetyl-Cytidine (11)

Triethylamine (6.35 mL, 45.55 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (5.66 g, 81.99 mmol) and phosphorous oxychloride (0.86 mL, 9.11 mmol) in 50 mL of anhydrous acetonitrile. To the
10 resulting suspension a solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-C-allyl uridine (2.32 g, 4.55 mmol) in 30 mL of acetonitrile was added dropwise and the reaction mixture was stirred for 4 h at room temperature. The reaction was concentrated *in vacuo* to a minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq.
15 sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The resulting foam was dissolved in 50 mL of 1,4-dioxane and treated with 29% aq. NH₄OH overnight at room temperature. TLC (chloroform:methanol / 9:1) showed complete conversion of the starting material. The solution was evaporated, dried by
20 coevaporation with anhydrous pyridine and acetylated with acetic anhydride (0.52 mL, 5.46 mmol) in pyridine overnight. The reaction mixture was quenched with methanol, evaporated, the residue was dissolved in chloroform, washed with sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, evaporated to dryness and purified by flash chromatography on silica gel (3% MeOH in chloroform). Yield 2.3 g (90%) as
25 a white foam.

Example 40: 5'-O-Dimethoxytrityl-2'-C-Allyl-N⁴-Acetyl-Cytidine

This compound was obtained analogously to the uridine derivative 9 in 55% yield.

Example 41: 5'-O-Dimethoxytrityl-2'-C-allyl-N⁴-Acetyl-Cytidine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (12)

2'-O-Dimethoxytrityl-2'-C-allyl-N⁴-acetyl cytidine (0.8 g, 1.31 mmol) was dissolved in dry dichloromethane under argon. N,N-Diisopropylethylamine
5 (0.46 mL, 2.62 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.38 mL, 1.7 mmol) was added dropwise to a stirred reaction solution and stirring was continued for 2 h at room temperature. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was
10 concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using chloroform:ethanol / 98:2 with 2% triethylamine mixture as eluent. Yield: 0.91 g (85%), white foam.

Example 42: 2'-Deoxy-2'-Methylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine
15 14 (Hansske,F.; Madej,D.; Robins,M. J. *Tetrahedron* **1984**, *40*, 125 and Matsuda,A.; Takenuki,K.; Tanaka,S.; Sasaki,T.; Ueda,T. *J. Med. Chem.* **1991** *34*, 812) (2.2 g, 4.55 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column.
20 2'-Deoxy-2'-methylene-uridine (1.0 g, 3.3 mmol, 72.5%) was eluted with 20% MeOH in CH₂Cl₂.

Example 43: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine (15)

2'-Deoxy-2'-methylene-uridine (0.91 g, 3.79 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added
25 dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column
30 using EtOAc:hexanes as eluant to yield 15 (0.43 g, 0.79 mmol, 22%).

Example 44: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (17)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.43 g, 0.8 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.28 mL, 1.6 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.25 mL, 1.12 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.3 g, 0.4 mmol, 50%) was purified by flash column chromatography over silica gel using a 25-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.42 (CH₂Cl₂: MeOH / 15:1)

Example 45: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine **14** (1.92 g, 12.6 mmol) and triphenylphosphine (2.5 g, 9.25 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol, 70%) eluted with 25% hexanes in EtOAc.

Example 46: 2'-Deoxy-2'-Difluoromethylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol, 68%) was eluted with 20% MeOH in CH₂Cl₂.

Example 47: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine (16)

2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.42 g, 4.18 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT
5 for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 40% EtOAc:hexanes as eluant to yield 5'-O-DMT-2'-deoxy-2'-
10 difluoromethylene-uridine **16** (1.05 g, 1.8 mmol, 45%).

Example 48: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (18)

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.577 g, 1 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a
15 round-bottom flask under Ar. Diisopropylethylamine (0.36 mL, 2 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.44 mL, 1.4 mmol). The reaction mixture was stirred for 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.404 g, 0.52 mmol,
20 52%) was purified by flash chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂: MeOH / 15:1).

Example 49: 2'-Deoxy-2'-Methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-4-N-Acetyl-Cytidine 20

25 Triethylamine (4.8 mL, 34 mmol) was added to a solution of POCl₃ (0.65 mL, 6.8 mmol) and 1,2,4-triazole (2.1 g, 30.6 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) uridine **19** (1.65 g, 3.4 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at room temperature for 4 h. The
30 mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved in dioxane (10 mL) and aq.

ammonia (20 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (3 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5 mL). The mixture was
5 concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetylcytidine 20 (1.3 g, 2.5 mmol, 73%) was eluted with 20% EtOAc in hexanes.

10 Example 50: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine 21

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetylcytidine 20 (1.3 g, 2.5 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was
15 triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-methylene-4-N-acetylcytidine (0.56 g, 1.99 mmol, 80%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-methylene-4-N-acetylcytidine (0.56 g, 1.99 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.81 g, 2.4 mmol) in pyridine (10 mL) was added dropwise over 15 m. The
20 resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using
25 EtOAc:hexanes / 60:40 as eluant to yield 21 (0.88 g, 1.5 mmol, 75%).

Example 51: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine 3'-(2-Cyanoethyl-N,N-diisopropylphosphoramidite) (22)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-N-acetylcytosine 21 (0.88 g, 1.5 mmol) dissolved in dry CH₂Cl₂ (10 mL) was
30 placed in a round-bottom flask under Ar. Diisopropylethylamine (0.8 mL, 4.5 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture

was stirred 2 h at room temperature and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product **22** (0.82 g, 1.04 mmol, 69%) was purified by flash chromatography over silica gel using 50-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.36 (CH₂Cl₂:MeOH / 20:1).

Example 52: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyl disiloxane-1,3-diyl)-4-N-Acetyl-Cytidine (24**)**

Et₃N (6.9 mL, 50 mmol) was added to a solution of POCl₃ (0.94 mL, 10 mmol) and 1,2,4-triazole (3.1 g, 45 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine **23** ([described in example 45] 2.6 g, 5 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at RT for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved in dioxane (20 mL) and aq. ammonia (30 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (5 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5mL). The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine **24** (2.2 g, 3.9 mmol, 78%) was eluted with 20% EtOAc in hexanes.

Example 53: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine (25**)**

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine **24** (2.2 g, 3.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol, 72%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-difluoromethylene-

4-*N*-acetyl-cytidine (0.89 g, 2.8 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.03 g, 3.1 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield 25 (1.2 g, 1.9 mmol, 68%).

Example 54: 1-(2'-Deoxy-2'-Difluoromethylene-5'-*O*-Dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-Acetylcytosine 3'-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) (26)

1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-acetylcytosine 25 (0.6 g, 0.97 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.5 mL, 2.9 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product 26, a white foam (0.52 g, 0.63 mmol, 65%) was purified by flash chromatography over silica gel using 30-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂:MeOH / 20:1).

Example 55: 2'-Keto-3',5'-*O*-(Tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine (28)

Acetic anhydride (4.6 mL) was added to a solution of 3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (Brown, J.; Christodolou, C.; Jones, S.; Modak, A.; Reese, C.; Sibanda, S.; Ubasawa A. *J. Chem. Soc. Perkin Trans. I* 1989, 1735) (6.2 g, 9.2 mmol) in DMSO (37 mL) and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated *in vacuo*. The residue was taken up in EtOAc and washed with water. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified on a silica gel column to yield 2'-keto-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine 28 (4.8 g, 7.2 mmol, 78%).

Example 56: 2'-Deoxy-2'-methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)

Under a pressure of argon, sec-butyllithium in hexanes (11.2 mL, 14.6 mmol) was added to a suspension of triphenylmethylphosphonium iodide (7.07 g, 17.5 mmol) in THF (25 mL) cooled at -78 °C. The homogeneous orange solution was allowed to warm to -30 °C and a solution of 2'-keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 28 (4.87 g, 7.3 mmol) in THF (25 mL) was transferred to this mixture under argon pressure. After warming to RT, stirring was continued for 24 h. THF was evaporated and replaced by CH₂Cl₂ (250 mL), water was added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H₂O (20 mL), 5% aqueous NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give the crude compound, which was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 29 (3.86 g, 5.8 mmol, 79%).

Example 57: 2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (3.86 g, 5.8 mmol) dissolved in THF (30 mL) was treated with 1 M TBAF in THF (15 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (1.8 g, 4.3 mmol, 74%) was eluted with 10% MeOH in CH₂Cl₂.

Example 58: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)

2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (0.75 g, 1.77 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.66 g, 1.98 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in

CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as an eluant to yield 29 (0.81 g, 1.1 mmol, 62%).

5 Example 59: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (31)

1-(2'-Deoxy-2'-methylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-6-*N*-(4-*t*-butylbenzoyl)-adenine 29 dissolved in dry CH₂Cl₂ (15 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine was added, followed by
10 the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite. The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product was purified by flash chromatography over silica gel using 30-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant (0.7 g, 0.76
15 mmol, 68%). R_f 0.45 (CH₂Cl₂: MeOH / 20:1)

Example 60: 2'-Deoxy-2'-Difluoromethylene-3',5'-*O*-(Tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine

2'-Keto-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine 28 (6.7 g, 10 mmol) and triphenylphosphine (2.9 g, 11 mmol) were
20 dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate (2.3 g, 15 mmol) in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and
25 chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (4.1g, 6.4 mmol, 64%) eluted with 15% hexanes in EtOAc.

Example 61: 2'-Deoxy-2'-Difluoromethylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine

30 2'-Deoxy-2'-difluoromethylene-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (4.1 g, 6.4 mmol) dissolved in THF (20 mL)

was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol, 77%) was eluted with 20% MeOH in CH₂Cl₂.

5 Example 62: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine (30)

2'-Deoxy-2'-difluoromethylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as eluant to yield 30 (2.6 g, 3.41 mmol, 69%).

Example 63: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (32)

1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-6-*N*-(4-*t*-butylbenzoyl)-adenine 30 (2.6 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (25 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (1.06 mL, 4.76 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). 32 (2.3 g, 2.4 mmol, 70%) was purified by flash column chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.52 (CH₂Cl₂: MeOH / 15:1).

Example 64: 2'-Deoxy-2'-Methoxycarbonylmethylidene-3',5'-*O*-(Tetraiso-propyldisiloxane-1,3-diyl)-Uridine (33)

Methyl(triphenylphosphoranylidene)acetate (5.4 g, 16 mmol) was added to a solution of 2'-keto-3',5'-*O*-(tetraisopropyl disiloxane-1,3-diyl)-uridine 14 in

- CH₂Cl₂ under argon. The mixture was left to stir at RT for 30 h. CH₂Cl₂ (100 mL) and water were added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H₂O (20 mL), 5% aq. NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give crude product, that was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methoxycarbonylmethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5.8 g, 10.8 mmol, 67.5%).

Example 65: 2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (34)

- Et₃N•3 HF (3 mL) was added to a solution of 2'-deoxy-2'-methoxycarbonylmethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5 g, 9.3 mmol) dissolved in CH₂Cl₂ (20 mL) and Et₃N (15 mL). The resulting mixture was evaporated *in vacuo* after 1 h and chromatographed on a silica gel column eluting 2'-deoxy-2'-methoxycarbonylmethylidene-uridine **34** (2.4 g, 8 mmol, 86%) with THF:CH₂Cl₂ / 4:1.

Example 66: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (35)

- 2'-Deoxy-2'-methoxycarbonylmethylidene-uridine **34** (1.2 g, 4.02 mmol) was dissolved in pyridine (20 mL). A solution of DMT-Cl (1.5 g, 4.42 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 2-5% MeOH in CH₂Cl₂ as an eluant to yield 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine **35** (2.03 g, 3.46 mmol, 86%).

Example 67: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (36)

- 1-(2'-Deoxy-2'-2'-methoxycarbonylmethylidene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uridine **35** (2.0 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (1.2 mL,

6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.91 mL, 4.08 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). 5'-*O*-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine 3'-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) **36** (1.8 g, 2.3 mmol, 67%) was purified by flash column chromatography over silica gel using a 30-60% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. *R*_f 0.44 (CH₂Cl₂:MeOH / 9.5:0.5).

Example 68: 2'-Deoxy-2'-Carboxymethylidene-3',5'-*O*-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine **37**

2'-Deoxy-2'-methoxycarbonylmethylidene-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5.0 g, 10.8 mmol) was dissolved in MeOH (50 mL) and 1 N NaOH solution (50 mL) was added to the stirred solution at RT. The mixture was stirred for 2 h and MeOH removed *in vacuo*. The pH of the aqueous layer was adjusted to 4.5 with 1N HCl solution, extracted with EtOAc (2 x 100 mL), washed with brine, dried over MgSO₄ and concentrated *in vacuo* to yield the crude acid. 2'-Deoxy-2'-carboxymethylidene-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-uridine **37** (4.2 g, 7.8 mmol, 73%) was purified on a silica gel column using a gradient of 10-15% MeOH in CH₂Cl₂.

Example 69: Synthesis of 2'-C-allyl-U phosphoramidite from 5'-*O*-DMT-3'-*O*-TBDMS-Uridine

Referring to Figure 54, in order to simplify the synthetic scheme for phosphoramidites **5** and **8** we also explored the potential of 5'-*O*-DMT-3'-*O*-TBDMS-Uridine **10** (side product in preparation of standard RNA monomers) as a starting material in the synthesis of key intermediate **4**. Phenoxythiocarbonylation of starting synthon **10** according to Robins (Robins, M. J., Wilson J. S. and Hansske, F. (1983), *J. Am. Chem. Soc.*, 105, 4059) surprisingly led to thioester **11** (91 %) without noticeable migration (Scaringe, S.A., Franclyn, C. & Usman, N. (1990) *Nucleic Acids Res.*, 18, 5433-5441) of the TBDMS group. Comparative analysis of ¹H NMR data for compounds **10** and **11** revealed that resonance of H-2' experienced up field shift of 2,0 ppm (from 6,06 to 4,13) in **11** compare to starting compound **10**, at the same time chemical shift of H-3' and H-1' changed only slightly: 4.83 ppm(H-3') and

6.48 ppm (H-1') in 11 compare to 4.36(H-3') ppm and 5.93 ppm (H-1') in 10 and chemical shift of H-4' remains practically unchanged indicating acylation at C-2-OH. Heck allylation of intermediate 11 with 2-,2'-Azobis-(2-methyl propionitrile) (other groups can be introduced by standard procedures) resulted in a formation of 2'-C-allyl derivative 12 (70 %) and related 2'-deoxy by-product (15%). Subsequent desilylation of 12 led to 5'-O-DMT derivative 4 identical to the one synthesized from thioester 2. Since the starting material for this route is commercially available this may represent a less laborious way to key synthon 4 as well as for other 2'- modified monomers. This methodology can be used to introduce other 2'-C-allyl groups using compound 11 (or its equivalent for other bases) as an intermediate.

Example 70: Synthesis of 5'-O-Dimethoxytrityl-2'-O-Phenoxythiocarbonyl-3'-O-t-butylidimethylsilyl-uridine 11.

To a stirred solution of 5'-O-Dimethoxytrityl-3'-O-t-butylidimethylsilyl-uridine (Commercially available from Chem Genes Corporation) (5,0 g 7,57 mmol) and dimethylaminopyridine (1,8g, 15 mmol) in 100 ml of dry acetonitril a solution of phenylchlorothionoformate (1.26ml, 9,1 mmol) in 25 ml of acetonitrile was added dropwise and the reaction mixture stirred at room temperature for 3 hours. TLC (ethylacetate-hexanes 1:1) showed disappearance of starting material and the reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel CH₂Cl₂ as an eluent to give 5.51g (91.3%) of the product.

¹H NMR (CDCl₃) δ 0.95 (s, 9H, tBu), 0.11 (s, 3H, CH₃), 0.04 (s, 3H, CH₃) 3.57 (2H, H5', H5'', m J5',4'=2.4., J5'',4'=2,8., J5',5'=11.0), 3.86 (6H, OCH₃, s), 4.07 (1H, H4', m), 4.83 (1H, H3', dd, J3',4'=2,8 J3',2'=5,2), 5.44 (1H, H5, d, J5,6=8.0) 5.99 (1H, H2', dd, J2',1'=6.4 , J2',3'= 5,2), 6.46 (1H, H1', d, J1',2'=6.4) , 6.89-7.79 (18H, DMT, Phe, m), 7.88 (1H, H6, d, J6,5=8.0), 7.95 (1H, N-H, bs).

Example 71: Synthesis of 5'-O-Dimethoxytrityl-2'-C-Allyl-3'-O-t-butylidimethylsilyl-uridine(12)

To a refluxing under argon solution of 5'-O-Dimethoxytrityl-2'-O-Phenoxythiocarbonyl-3'-O-t-butylidimethylsilyl-uridine (5,5g, 6,9 mmol) and

allyltributyltin (10,7ml, 34,5 mmol) in dry toluene (150 ml) a solution of 2-,2'-Azobis-(2-methyl propionitrile) (0.28g 1,72 mmol) in 50 ml of dry toluene was added dropwise for 1 hour. The resulting mixture was allowed to reflux under argon for additional 2 hours. After that it was concentrated in vacuo and
 5 purified by flash chromatography on silica gel with gradient ethylacetate in hexanes (0-30%) as an eluent. Yield 3.38g (70.0%).

¹H NMR (CDCl₃) δ 0.95 (s, 9H, tBu), 0.11 (s, 3H, CH₃), 0.04 (s, 3H, CH₃), 2.23 (1H, H6', m), . 2.38-2.52 (2H, H6" and H2', m), 3.46 (2H, H5' and H5", m, J_{5',4'}=2.5., J_{5'',4'}=3.2 J_{5',5'}=10.8), 3.86 (6H, OCH₃, s), 4.13 (1H,
 10 H4', dd, J_{4',3'}=8.0, J_{4',5'}=3.2, J_{4',5'}=2.5), 4.46 (1H, H3', m), 5.15 (1H, H8', d, J_{8',7'}=10.0), 5.20 (1H, H9', d, J_{9',7'}=17.3), 5.44 (1H, H5, d, J_{5,6}=8.0), 5.81 (1H, H7', dddd, J_{7',6'}=6.0, J_{7',6''}=8.0), 6.14 (1H, H1', d, J_{1',2'}=8.0), 6.88-7.52 (13H, DMT, m), 7.76 (1H, H6, d, J_{6,5}=8.0), 8.17 (1H, N-H, bs)

Example 72: Synthesis of 5'-O-Dimethoxytrityl-2'-C-Allyl Uridine (4) from 5'-O-Dimethoxytrityl-2'-C-Allyl-3'-O-t-butylidimethyl-silyl-uridine (12).
 15

Standard deprotection of TBDMS derivative 12 utilizing general method A furnished product 4 (yield 80%) identical to the compound prepared from 2'-C-allyl derivative 3.

Uses

20 The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan *et al.* PCT WO
 25 94/02595.

The following are non-limiting examples showing the synthesis of nucleic acids using 2'-O-methylthioalkyl-substituted phosphoramidites and the syntheses of the amidites.

Example 73: Synthesis of Hammerhead Ribozymes Containing 2'-O-alkylthioalkylnucleotides & Other Modified Nucleotides

The method of synthesis follows the procedure for normal RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J.
5 *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* **1990**, *18*, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. These 2'-O-alkylthioalkyl substituted phosphoramidites may be incorporated not only into hammerhead
10 ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 74: Synthesis of base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) nucleosides (2)

15 Referring to Figure 55, standard introduction of "Markiewicz" protecting group to the base-protected nucleosides according to "Oligonucleotides and Analogues. A Practical Approach", ed. F. Eckstein, IRL Press, 1991 resulted in protected nucleosides (2) with 85-100% yields. Briefly, in a non-limiting
20 example, Uridine (20g, 81.9 mmol) was dried by two coevaporations with anhydrous pyridine and re dissolved in the anhydrous pyridine. The above solution was cooled (0°C) and solution of 1,3-dichloro-1,1,3,3-tetraisopropylsiloxane (28.82 mL, 90.09 mmol) in 30 mL of anhydrous dichloroethane was added dropwise under stirring. After the addition was
25 completed the reaction mixture was allowed to warm to room temperature and stirred for additional two hours. Then it was quenched with MeOH (25 mL) and evaporated to dryness. The residue was dissolved in methylene chloride and washed with saturated NaHCO₃ and brine. The organic layer was evaporated to dryness and then coevaporated with toluene to remove traces of pyridine to give 39g (98%) of compound 2 (B=Ura) which was used without
30 further purification.

Other 3',5'-O-(tetraisopropylidisiloxane-1,3-di-yl)- nucleosides were obtained in 75-90% yields, using the protocol described above, starting from

base-protected nucleosides with final purification of the products by flash chromatography on silica gel when necessary.

Example 75: General procedure for the synthesis of 2'-O-methylthiomethyl nucleosides (3)

- 5 Referring to Figure 55, to a stirred ice-cooled solution of the mixture of base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) nucleoside (2) (7 mmol), methyl disulfide (70 mmol), 2,6-lutidine (7 mmol) in methylene chloride (100 mL) or mixture methylene chloride - acetonitrile (1:1) under positive pressure of argon, solution of benzoyl peroxide (28 mmol) in methylene
10 chloride was added dropwise during 1 hour. After complete addition the reaction mixture was stirred at 0°C under argon for additional 1 hour. The solution was allowed to warm to room temperature, diluted with methylene chloride (100 mL), washed twice with saturated aq NaHCO₃ and brine. The organic layer was dried over sodium sulfate and evaporated to dryness. The
15 residue was purified by flash chromatography on silica using 1-2% methanol in methylene chloride as an eluent to give corresponding methylthiomethyl nucleosides with 55-70% yield.

Example 76: 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides. (6)

- Method A. The solution of the base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-2'-O-methylthiomethyl nucleoside (3) (2.00
20 mmol) in 10 ml of dry tetrahydrofuran (THF) was treated with 1M solution of tetrabutylammoniumfluoride in THF (3.0 ml) for 10-15 minutes at room temperature. Resulting mixture was evaporated, the residue was loaded to the silica gel column, washed with 1L of chloroform, and the desired
25 deprotected compound was eluted with 5-10% methanol in dichloromethane. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The
30 reaction was quenched with methanol (20 ml), evaporated, dissolved in chloroform, washed with saturated aq sodium bicarbonate and brine. Organic layer was dried over sodium sulfate and evaporated. The residue was purified

by flash chromatography on silica gel to give 5'-O-Dimethoxytrityl derivatives with 70-80% yield.

Method B. Alternatively, 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides (6) may also be synthesized using 5'-O-Dimethoxytrityl-3'-O-t-Butyl-dimethylsilyl Nucleosides (4) as the starting material. Compound 4 is commercially available as a by-product during RNA phosphoramidite synthesis. Compound 4 is converted into 3'-O-t-butyl dimethylsilyl-2'-O-methylthiomethyl nucleoside 5, as described under example 3. The solution of the base-protected 3'-O-t-butyl dimethylsilyl-2'-O-methylthiomethyl nucleoside 5 (2.00 mmol) in 10 ml of dry tetrahydrofuran (THF) was treated with 1M solution of tetrabutylammoniumfluoride in THF (3.0 ml) for 10-15 minutes at room temperature. The resulting mixture was evaporated, and purified by flash silica gel chromatography to give nucleosides 6 in 90% yield.

Example 77: 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides-3'-(2-Cyanoethyl-N,N-diisopropylphosphoramidites) (7)

Standard phosphorylation of nucleoside 6 according to Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* **1990**, *18*, 5433-5441 yielded phosphoramidites in 70-85% yield.

Example 78: General procedure for the synthesis of 2'-O-Methylthiophenyl nucleosides

To a stirred ice-cooled solution of the mixture of base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) nucleoside (14.7 mmol), thioanisole (147 mmol), N,N-dimethylaminopyridine (58.8 mmol) in acetonitrile (100 mL) under positive pressure of argon, benzoyl peroxide (36.75 mmol) was added portionwise over 3 hours. After complete addition the reaction mixture was allowed to warm to room temperature and was stirred under argon for an additional 1 hour. The solvents were removed in vacuo, the residue was dissolved in ethylacetate, washed twice with saturated aq NaHCO₃ and brine. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica using mixture EtOAc-hexanes (1:1) as eluent to give the corresponding methylthiophenyl nucleosides with 55-65% yield.

Example 79: 5'-O-Dimethoxytrityl-2'-O-Methylthiophenyl-Nucleosides.

These compounds were prepared as described above under examples 76 and 76.

Example 80: 5'-O-Dimethoxytrityl-2'-O-Methylthiophenyl-Nucleosides-3'-(2-Cyanoethyl N,N-diisopropylphosphoramidites)

Standard phosphitylation according to Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidites in 70-85% yield.

Example 81: Ribozymes containing 2'-O-methylthiomethyl substitutions

10 In a non-limiting example 2'-O-methylthioalkyl substitutions were made at various positions within a hammerhead ribozyme motif (Fig. 56, including U4 and U7 positions). The target site B was targeted by the hammerhead ribozyme in this non-limiting example.

15 Hammerhead ribozymes (see Fig. 56) were synthesized using solid-phase synthesis, as described above. Several positions were modified, individually or in combination, with 2'-O-methylthiomethyl groups.

RNA cleavage assay *in vitro*:

20 Substrate RNA is 5' end-labeled using [γ - 32 P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂. The reaction is
25 initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of
30 time.

Referring to Figure 57, hammerhead ribozymes containing 2'-O-methylthiomethyl modifications at various positions cleave the target RNA efficiently. Surprisingly, all the 2'-O-methylthiomethyl -substituted ribozymes cleaved the target RNA more efficiently compared to the control hammerhead ribozyme.

Sequences listed in Figure 56 and the modifications described in Figure 56 and 57 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other combinations of 2'-hydroxyl group modifications can be readily generated using techniques known in the art, and are within the scope of the present invention.

The following are non-limiting examples showing the synthesis of non-nucleotide mimetic-containing catalytic nucleic acids using non-nucleotide phosphoramidites.

Such non-nucleotides can be located in the binding arms, core or the loop adjacent stem II of a hammerhead type ribozyme. Those in the art following the teachings herein can determine optimal locations in these regions. Surprisingly, abasic moieties can be located in the core of such a ribozyme.

Example 82: Synthesis of Abasic nucleotides

The synthesis of 1-deoxy-D-ribofuranose phosphoramidite **9** is shown in Figure 58. Our initial efforts concentrated on the deoxygenation of synthon **1**, prepared by a "one pot" procedure from D-ribose. Phenoxythiocarbonylation of acetonide **1** under Robins conditions led to the β -anomer **2** ($J_{1,2} = 1.2$ Hz) in modest yield (45-55%). Radical deoxygenation using $\text{Bu}_3\text{SnH/AIBN}$ resulted in the formation of the ribitol derivative **3** in 50% yield. Subsequent deprotection with 90% CF_3COOH (10 m) and introduction of a dimethoxytrityl group led to the key intermediate **4** in 40% yield (Yang et al., *Biochemistry* 1992, 31, 5005-5009; Perreault et al., *Biochemistry* 1991, 30, 4020-4025; Paoletta et al., *EMBO J.* 1992, 11, 1913-1919; Peiken et al., *Science* 1991, 253, 314-317).

The low overall yield of this route prompted us to investigate a different approach to **4** (Fig. 58). Phenylthioglycosides, successfully employed in the Keck reaction, appeared to be an alternative. However, it is known that free-radical reduction of the corresponding glycosyl bromides with participating acyl groups at the C2-position can result in the migration of the 2-acyl group to the C1-position (depending on Bu_3SnH concentration). Therefore we subjected phenylthioglycoside **5** to radical reduction with Bu_3SnH (6.1 eq.) in the presence of Bz_2O_2 (2 eq.) resulting in the isolation of tribenzoate **6** in 63% yield (Fig. 9B). Subsequent debenzoylation and dimethoxytritylation led to synthon **4** in 70% yield. Introduction of the TBDMS group, using standard conditions, resulted in the formation of a 4:1 ratio of 2- and 3-isomers **8** and **7**. The two regioisomers were separated by silica gel chromatography. The 2-*O*-*t*-butyldimethylsilyl derivative **8** was phosphitylated to provide phosphoramidite **9** in 82% yield.

15 Example 83: RNA cleavage assay *in vitro*

Ribozymes and substrate RNAs were synthesized as described above. Substrate RNA was 5' end-labeled using $[\gamma\text{-}^{32}\text{P}]$ ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme were denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate were incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl_2 . The reaction was initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μl are taken at regular intervals of time and the reaction quenched by mixing with an equal volume of 2X formamide stop mix. The samples were resolved on 20 % denaturing polyacrylamide gels. The results were quantified and percentage of target RNA cleaved is plotted as a function of time.

30 Referring to Figure 59 there is shown the general structure of a hammerhead ribozyme targeted against site B (HH-B) with various bases numbered. Various substitutions were made at several of the nucleotide positions in HH-B. Specifically referring to Figure 60, substitutions were made

at the U4 and U7 positions marked as X4 and X7 and also in loop II in the positions marked by an X. The RNA cleavage activity of these substituted ribozymes is shown in the following figures. Specifically, Figure 61 shows cleavage by an abasic substituted U4 and an abasic substituted U7. As will
5 be noted, abasic substitution at U4 or U7 does not significantly affect cleavage activity. In addition, inclusion of all abasic moieties in stem II loop does not significantly reduce enzymatic activity as shown in Figure 62. Further, inclusion of a 3' inverted deoxyribose does not inactivate the RNA cleavage activity as shown in Figure 63.

10 Example 84: Smooth Muscle Cell Proliferation Assay

Hammerhead ribozyme (HH-A) is targeted to a unique site (site A) within *c-myb* mRNA. Expression of *c-myb* protein has been shown to be essential for the proliferation of rat smooth muscle cell (Brown et al., 1992 *J. Biol. Chem.* 267, 4625).

15 The ribozymes that cleaved site A within *c-myb* RNA described above were assayed for their effect on smooth muscle cell proliferation. Rat vascular smooth muscle cells were isolated and cultured as described (Stinchcomb et al., *supra*). These primary rat aortic smooth muscle cells (RASMC) were plated in a 24-well plate (5×10^3 cells/well) and incubated at 37°C in the
20 presence of Dulbecco's Minimal Essential Media (DMEM) and 10% serum for ~16 hours.

These cells were serum-starved for 48-72 hours in DMEM (containing 0.5% serum) at 37°C. Following serum-starvation, the cells were treated with lipofectamine (LFA)-complexed ribozymes (100 nM ribozyme was complexed
25 with LFA such that LFA:ribozyme charge ration is 4:1).

Ribozyme:LFA complex was incubated with serum-starved RASMC cells for four hours at 37°C. Following the removal of ribozyme:LFA complex from cells (after 4 hours), 10% serum was added to stimulate smooth cell proliferation. Bromo-deoxyuridine (BrdU) was added to stain the cells. The
30 cells were stimulated with serum for 24 hours at 37°C.

Following serum-stimulation, RASMC cells were quenched with hydrogen peroxide (0.3% H₂O₂ in methanol) for 30 min at 4°C. The cells were then denatured with 0.5 ml 2N HCl for 20 min at room temperature. Horse serum (0.5 ml) was used to block the cells at 4°C for 30 min up to ~16
5 hours.

The RASMC cells were stained first by treating the cells with anti-BrdU (primary) antibody at room temperature for 60 min. The cells were washed with phosphate-buffered saline (PBS) and stained with biotinylated affinity-purified anti-mouse IgM (Pierce, USA) secondary antibody. The cells were
10 counterstained using avidin-biotinylated enzyme complex (ABC) kit (Pierce, USA).

The ratio of proliferating:non-proliferating cells was determined by counting stained cells under a microscope. Proliferating RASMCs will incorporate BrdU and will stain brown. Non-proliferating cells do not
15 incorporate BrdU and will stain purple.

Referring to Figure 64 there is shown a ribozyme which cleaves the site referred to as HH-A. Substitutions of abasic moieties in place of U4 as shown in Figure 65 provided active ribozyme as shown in Figure 66 using the above-noted rat aortic smooth muscle cell proliferation assay.

20 The method of this invention generally features HPLC purification of ribozymes. An example of such purification is provided below in which a synthetic ribozyme produced on a solid phase is blocked. This material is then released from the solid phase by a treatment with methanolic ammonia, subsequently treated with tetrabutylammonium fluoride, and purified on
25 reverse phase HPLC to remove partially blocked ribozyme from "failure" sequences. Such "failure" sequences are RNA molecules which have a nucleotide base sequence shorter to that of the desired enzymatic RNA molecule by one or more of the desired bases in a random manner, and possess free terminal 5'-hydroxyl group. This terminal 5'-hydroxyl in a
30 ribozyme with the correct sequence is still blocked by lipophilic dimethoxytrityl group. After such partially blocked enzymatic RNA is purified, it is deblocked by a standard procedure, and passed over the same or a similar HPLC

reverse phase column to remove other contaminating components, such as other RNA molecules or nucleotides or other molecules produced in the deblocking and synthetic procedures. The resulting molecule is the native enzymatically active ribozyme in a highly purified form.

- 5 Below are provided examples of such a method. These examples can be readily scaled up to allow production and purification of gram or even kilogram quantities of ribozymes.

Example 85: HPLC Purification, Reverse-Phase

- 10 In this example solid phase phosphoramidite chemistry was employed for synthesis of a ribozyme. Monomers used were 2'-*t*-butyl-dimethylsilyl cyanoethylphosphoramidites of uridine, *N*-benzoyl-cytosine, *N*-phenoxyacetyl adenosine, and guanosine (Glen Research, Sterling, VA).

- 15 Solid phase synthesis was carried out on either an ABI 394 or 380B DNA/RNA synthesizer using the standard protocol provided with each machine. The only exception was that the coupling step was increased from 10 to 12 minutes. The phosphoramidite concentration was 0.1 M. Synthesis was done on a 1 μ mol scale using a 1 μ mol RNA reaction column (Glen Research). The average coupling efficiencies were between 97% and 98% for the 394 model and between 97% and 99% for the 380B model, as
20 determined by a calorimetric measurement of the released trityl cation. The final 5'-DMT group was not removed.

- 25 After synthesis, the ribozymes were cleaved from the CPG support, and the base and phosphotriester moieties were deprotected in a sterile vial by incubation in dry ethanolic ammonia (2 mL) at 55 °C for 16 hours. The reaction mixture was cooled on dry ice. Later, the cold liquid was transferred
into a sterile screw cap vial and lyophilized.

- 30 To remove the 2'-*t*-butyldimethylsilyl groups from the ribozyme the obtained residue was suspended in 1 M tetra-*n*-butylammonium fluoride in dry THF (TBAF), using a 20-fold excess of the reagent for every silyl group, for 16 hours at ambient temperature. The reaction was quenched by adding an

equal volume of a sterile 1 M triethylamine acetate, pH 6.5. The sample was cooled and concentrated on a SpeedVac to half of the initial volume.

The ribozymes were purified in two steps by HPLC on a C4 300 Å 5 µm DeltaPak column in an acetonitrile gradient.

- 5 The first step, or "trityl on" step, was a separation of 5'-DMT-protected ribozyme(s) from failure sequences lacking a 5'-DMT group. Solvents used for this step were: A (0.1 M triethylammonium acetate, pH 6.8) and B (acetonitrile). The elution profile was: 20% B for 10 minutes, followed by a linear gradient of 20% B to 50% B over 50 minutes, 50% B for 10 minutes, a
10 linear gradient of 50% B to 100% B over 10 minutes, and a linear gradient of 100% B to 0% B over 10 minutes.

- The second step was a purification of a completely deprotected, *i.e.* following the removal of the 5'-DMT group, ribozyme by a treatment with 2% trifluoroacetic acid or 80% acetic acid on a C4 300 Å 5 µm DeltaPak column in
15 an acetonitrile gradient. Solvents used for this second step were: A (0.1 M Triethylammonium acetate, pH 6.8) and B (80% acetonitrile, 0.1 M triethylammonium acetate, pH 6.8). The elution profile was: 5% B for 5 minutes, a linear gradient of 5% B to 15% B over 60 minutes, 15% B for 10 minutes, and a linear gradient of 15% B to 0% B over 10 minutes.

- 20 The fraction containing ribozyme, which is in the triethylammonium salt form, was cooled and lyophilized on a SpeedVac. Solid residue was dissolved in a minimal amount of ethanol and ribozyme in sodium salt form was precipitated by addition of sodium perchlorate in acetone. (K^+ or Mg^{2+} salts can be produced in an equivalent manner.) The ribozyme was collected
25 by centrifugation, washed three times with acetone, and lyophilized.

Example 86: RNA and Ribozyme Deprotection of Exocyclic Amino Protecting Groups Using ethylamine (EA)

- The polymer-bound oligonucleotide, either trityl-on or off, was suspended in a solution of ethylamine (EA) @ 25-55 °C for 10-30 min to remove the
30 exocyclic amino protecting groups (see Figure 67). The supernatant was removed from the polymer support. The support was washed with 1.0 mL of

EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder.

5 Table EVII is a summary of the results obtained using the improvements outlined in this application for base deprotection. From this data it is evident EA at 55° for 10 m or 40° for 10 m is efficient. The HPLC peak structure is almost identical between these schemes, and the yield for the ethylamine deprotected oligos is actually slightly better than the methylamine.

10 The second step of the deprotection of RNA molecules may be accomplished by removal of the 2'-hydroxyl alkylsilyl protecting group using TBAF for 8-24 h (Usman *et al. J. Am. Chem. Soc.* 1987, 109, 7845-7854). Applicant has determined that the use of anhydrous TEA•HF in *N*-methylpyrrolidine (NMP) for 0.5-1.5 h @ 55-65 °C gives equivalent or better results.

15 The following are examples of preferred embodiments of the present invention. Those in the art will recognize that these are not limiting examples but rather are provided to guide those in the art to the full breadth of meaning of the present invention. Routine procedures can be used to utilize other coupling regions not exemplified below.

20 Ribozymes were synthesized in two parts and tested without ligation for catalytic activity. Referring to Fig. 72, the cleavage activity of the half ribozymes containing between 5 and 8 base pairs stem IIs at 40 nM under single turnover conditions was comparable to that of the full length oligomer as shown in Figs. 73 and 74. The same half ribozymes were synthesized with
25 suitable modifications at the nascent stem II loop to allow for crosslinking. The halves were purified and chemically ligated, using a variety of crosslinking methods. The resulting full length ribozymes (see Fig. 71) exhibited similar cleavage activity as the linearly synthesized full length oligomer as shown in Fig. 74.

Example 87

Referring to Fig. 70 the 5' half of a hammerhead ribozyme was provided with a ribose group. This was oxidatively cleaved with NaIO_4 and reacted with the 3' half of the ribozyme having an amino group under reducing conditions.

- 5 The resulting ribozyme consisted of the two half ribozyme linked by a morpholino group.

- One equivalent of (200 micrograms) of 5' half hammerhead with a 3'OH and 5 equivalents (1000 micrograms) of 3' half with 5' C5-NH₂ all with HH-A were used in this reaction. The limiting oligonucleotide was oxidized first with
- 10 3.6 equivalents of sodium periodate for sixty minutes on ice in DEPC water quenched with 7.2 equivalents of ethylene glycol for 30 minutes on ice and the 5 equivalents of the amino oligo added. 0.5 Molar tricine buffer, pH 9, was added to provide 25 millimolar final tricine concentration and left for 30 minutes on ice. 50 equivalents of sodium cyanoborohydride was then added
- 15 and the pH reduced to 6.5 with acetic acid and reaction left for 60 minutes on ice. The resulting full length ribozyme was then purified for further analysis.

Example 88: Amide Bond

- Referring again to Fig. 70 and 71, a 5' half of ribozyme was provided with a carboxyl group at its 2' position and was coupled with an amine containing
- 20 3' half ribozyme. The provision of a coupling reagent resulted in a full-length ribozyme having an amide bond.

Example 89: Disulfide Bond

- Referring to Fig. 70 and 71, 250 micrograms of RPI3881 and 250 micrograms of RPI3636 half ribozyme were separately deprotected with
- 25 dithiothreitol overnight at 37°C. They were mixed together at 1:1 mole ratio in a 100 mM sodium phosphate buffer at pH 8 and 4M copper sulfate and 0.8 mM 1,10-phenanthroline (final concentrations) was added for two hours at room temperature (20-25°C) and the resulting mixture gel purified. The overall purification yield of full length ribozyme was 30%.

- 30 To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 1.8 KB region (containing site A) was synthesized by PCR using

primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed, using T7 RNA polymerase, in a standard transcription buffer in the presence of [α -³²P]CTP. The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol
5 followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (200 nM) and internally labeled 1.8 KB substrate RNA (<10 nM) were denatured and renatured separately in a standard
10 cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by
15 adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Few antiviral drug therapies are available that effectively inhibit established viral infections. Consequently, prophylactic immunization has
20 become the method of choice for protection against viral pathogens. However, effective vaccines for divergent viruses such as those causing the common cold, and HIV, the etiologic agent of AIDS, may not be feasible. Consequently, new antiviral strategies are being developed for combating viral infections.

25 Gene therapy represents a potential alternative strategy, where antiviral genes are stably transferred into susceptible cells. Such gene therapy approaches have been termed "intracellular immunization" since cells expressing antiviral genes become immune to viral infection (Baltimore, 1988 *Nature* 335, 395-396). Numerous forms of antiviral genes have been
30 developed, including protein-based antivirals such as transdominant inhibitory proteins (Malim et al., 1993 *J. Exp. Med.*, Bevec et al., 1992 *P.N.A.S. (USA)* 89, 9870-9874; Bahner et al., 1993 *J. Virol.* 67, 3199-3207) and viral-activated suicide genes (Ashorn et al., 1990 *P.N.A.S.(USA)* 87, 8889-8893). Although

effective in tissue culture, protein-based antivirals have the potential to be immunogenic *in vivo*. It is therefore conceivable that treated cells expressing such foreign antiviral proteins will be eradicated by normal immune functions. Alternatives to protein based antivirals are RNA based molecules such as antisense RNAs, decoy RNAs, agonist RNAs, antagonist RNAs, therapeutic editing RNAs and ribozymes. RNA is not immunogenic; therefore, cells expressing such therapeutic RNAs are not susceptible to immune eradication.

Example 90: Design and construction of U6-S35 Chimera

A transcription unit, termed **U6-S35**, is designed that contains the characteristic intramolecular stem of a **S35** motif (see Figure 76). As shown in Figure 77, 78 and 79 a desired RNA (e.g. ribozyme) can be inserted into the indicated region of U6-S35 chimera. This construct is under the control of a type 3 pol III promoter, such as a mammalian U6 small nuclear RNA (snRNA) promoter (see Fig. 75). U6-S35-HHI and U6-S35-HHII are non-limiting examples of the U6-S35 chimera.

As a non-limiting example, applicant has constructed a stable, active ribozyme RNA driven from a eukaryotic U6 promoter (Fig. 78). For stability, applicant incorporated a S35 motif as described in Fig. 76 and Fig. 77. A ribozyme sequence is inserted at the top of the stem, such that the ribozyme is separated from the S35 motif by an unstructured spacer sequence (Fig. 77, 78, 79). The spacer sequence can be customized for each desired RNA sequence. U6-S35 chimera is meant to be a non-limiting example and those skilled in the art will recognize that the structure disclosed in the figures 77, 78 and 79 can be driven by any of the known RNA polymerase promoters and are within the scope of this invention. All that is necessary is for the 5' region of a transcript to interact with its 3' region to form a stable intramolecular structure (S35 motif) and that the S35 motif is separated from the desired RNA by a stretch of unstructured spacer sequence. The spacer sequence appears to improve the effectiveness of the desired RNA.

By "unstructured" is meant lack of a secondary and tertiary structure such as lack of any stable base-paired structure within the sequence itself, and preferably with other sequences in the attached RNA.

By "spacer sequence" is meant any unstructured RNA sequence that separates the S35 domain from the desired RNA. The spacer sequence can be greater than or equal to one nucleotide.

In vitro Catalytic Activity of U6-S35-Ribozyme Chimeras:

5 U6-S35-HHI ribozyme RNA was synthesized using T7 RNA polymerase. HHI RNA was chemically synthesized using RNA phosphoramidite chemistry as described in Wincott et al., 1995 *Nucleic Acids Res.* The ribozyme RNAs were gel-purified and the purified ribozyme RNAs were heated to 55°C for 5 min. Target RNA used was ~650 nucleotide long. Internally-³²P-labeled
10 target RNA was prepared as described above. The target RNA was pre-heated to 37°C in 50 mM Tris.HCl, 10 mM MgCl₂ and then mixed at time zero with the ribozyme RNAs (to give 200 nM final concentration of ribozyme). At appropriate times an aliquot was removed and the reaction was stopped by
15 dilution in 95% formamide. Samples were resolved on a denaturing urea-polyacrylamide gel and products were quantitated on a phosphorimager®.

As shown in Figure 80, the U6-S35-HHI ribozyme chimera cleaved its target RNA as efficiently as a chemically synthesized HHI ribozyme. In fact, it appears that the U6-S35-HHI ribozyme chimera may be more efficient than the synthetic ribozyme.

20 *Accumulation of U6-S35-ribozyme transcripts*

An Actinomycin D assay was used to measure accumulation of the transcript in mammalian cells. Cells were transfected overnight with plasmids encoding the appropriate transcription units (2µg DNA/well of 6 well plate) using calcium phosphate precipitation method (Maniatis et al., 1982 *Molecular*
25 *Cloning* Cold Spring Harbor Laboratory Press, NY). After the overnight transfection, media was replaced and the cells were incubated an additional 24 hours. Cells were then incubated in media containing 5µg/ml Actinomycin D. At the times indicated, cells were lysed in guanidinium isothiocyanate, and total RNA was purified by phenol/chloroform extraction and isopropanol
30 precipitation as described by Chomczynski and Sacchi, 1987 *Anal. Biochem.*, 162, 156. RNA was analyzed by northern blot analysis and the levels of

specific RNAs were radioanalytically quantitated on a phosphorimager[®]. The level of RNA at time zero was set to be 100%.

As shown in Figure 81, the U6-S35-HHII ribozyme shown in Figure 79 is fairly stable in 293 mammalian cells with an approximate half-life of about 2
5 hours.

Example 91: Design and construction of VA1-S35 Chimera

Referring to Figure 83A, In order to express ribozymes from a VAI promoter, applicant has constructed a transcription unit consisting of a wild type VA1 sequence with two modifications: a "S35-like" motif extends from a
10 loop in the central domain (Figure 82); the 3' terminus is changed such that there is a more complete interaction between the 5' and the 3' region of the transcript (specifically, an "A-C" bulge is changed to an "A-U base pair and the termination sequence is part of the stem of S35 motif).

Accumulation of VA1-S35-ribozyme transcripts

15 An Actinomycin D assay was used to measure accumulation of the transcript in mammalian cells as described above. As shown in Figure 84, the VA1-S35-chimera, shown in Figure 83A, has approximately 10-fold higher stability in 293 mammalian cells compared to VA1-chimera, shown in Figure 25B that lacks the intramolecular S35 motif.

20 Besides ribozymes, desired RNAs like antisense, therapeutic editing RNAs, decoys, can be readily inserted into the indicated U6-S35 or VA1-S35 chimera to achieve therapeutic levels of RNA expression in mammalian cells.

Sequences listed in the Figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (mutations, insertions and
25 deletions) of the above examples can be readily generated using techniques known in the art, are within the scope of the present invention.

Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of

stromolysin, B7-1, B7-2, B7-3 and/or CD40 or other RNAs in a cell. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNAs associated with B7-1, B7-2, B7-3 and/or CD40 or other RNA related conditions. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and

- putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, B7-1, B7-2, B7-3 and/or CD40) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a
- 5 qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

Other embodiments are within the following claims.

10

TABLE I**Characteristics of Ribozymes****Group I Introns**

Size: ~200 to >1000 nucleotides.

Requires a U in the target sequence immediately 5' of the cleavage site.

Binds 4-6 nucleotides at 5' side of cleavage site.

Over 75 known members of this class. Found in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.

RNaseP RNA (M1 RNA)

Size: ~290 to 400 nucleotides.

RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.

Roughly 10 known members of this group all are bacterial in origin.

Hammerhead Ribozyme

Size: ~13 to 40 nucleotides.

Requires the target sequence UH immediately 5' of the cleavage site.

Binds a variable number nucleotides on both sides of the cleavage site.

14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figure 1)

Hairpin Ribozyme

Size: ~50 nucleotides.

Requires the target sequence GUC immediately 3' of the cleavage site.

Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent (Figure 3).

Hepatitis Delta Virus (HDV) Ribozyme

Size: 50 - 60 nucleotides (at present).

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Only 1 known member of this class. Found in human HDV (Figure 4).

***Neurospora* VS RNA Ribozyme**

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined. Only 1 known member of this class. Found in *Neurospora* VS RNA (Figure 5).

Table AII: Human Stromelysin Hammerhead Target Sequence

<u>nt</u> <u>Position</u>	<u>Sequence</u>	<u>SEQ. ID. NO.</u>
20	UAGAGCUAAGUAAAGCCAG	ID. NO. 01
126	ACACCAGCAUGAA	ID. NO. 02
147	AGAAAUUUCUAGA	ID. NO. 03
171	ACCUCAAAAAGAUUGUGAAACAGU	ID. NO. 04
240	AAUUGCAGAAGUUC	ID. NO. 05
287	GACACUCUGGAGGUGAUGCGCAAGCCCAGGUGU	ID. NO. 06
327	CUGAUGUUGGUCACUUCAGAAC	ID. NO. 07
357	GCAUCCCGAAGUGGAGGAAAACCCACCUUACAU	ID. NO. 08
402	AUUAUACACCAGAUUUGCCAAAAGAU	ID. NO. 09
429	CUGUUGAUUCUGCUGUUGAGA	ID. NO. 10
455	CUGAAAGUCUGGGAGAGGUGA	ID. NO. 11
513	CUGAUUAUAUGA	ID. NO. 12
592	UGCCUAUGCCCC	ID. NO. 13
624	AUGCCACUUUGAUGAUGAUGAACAAUGGACA	ID. NO. 14
679	AUUUCUCGUUGCUGCUCAUG	ID. NO. 15
725	CACUCAGCCAACACUGA	ID. NO. 16
801	AAGAUGAUUAUAAUGGCAUUCAGUCC	ID. NO. 17
827	CUCUAUGGACCUCUCCCCUGACUCCCCU	ID. NO. 18
859	CCCCUGGUACCCA	ID. NO. 19
916	UCCUGCUUUGUCCUUUGAUGCUGUCAGCAC	ID. NO. 20
958	AAUCCUGAUCUUUAAAGA	ID. NO. 21
975	CAGGCACUUUUGGCGCAAAUCCC	ID. NO. 22
1018	AUUGCAUUGAUCUCUUCALUUUGGCCAUC	ID. NO. 23
1070	GCAUAUGAAGUUA	ID. NO. 24
1203	AAAUUGAUGCAGCCAUUUCUGA	ID. NO. 25
1274	UUUGAUGAGAAGAGAAAUUCCAUGGAGC	ID. NO. 26
1302	CAGGCUUCCCCAAGCAAUAGCUGAAGAC	ID. NO. 27
1420	CCCAAUGCAAAG	ID. NO. 28
1485	AUGUAGAAGGCACAAUAUGGGCACUUUAAA	ID. NO. 29
1623	UCUUGCCGGUCAUUUUUAUGUUAU	ID. NO. 30
1665	GCUGCUGCUUAGC	ID. NO. 31

140

1733

CAACAGACAAGUGACUGUAUCU

ID. NO. 32

1769

CUUAUUUAUA

ID. NO. 33

Table AIII: Human Stromelysin HH Target Sequence

nt. Position	Target Sequence	Seq. ID. NO.
10	GCAAGGCAUA GAGACAACAUAGAGC	ID. NO. 34
21	GCAUAGAGACAACAU GAGCUAAGUAAAGCC	ID. NO. 35
27	AGACAACAUAGAGCUA AGUAAAGCCAGUGGA	ID. NO. 36
31	AACAUAGAGCUAAGUA AAGCCAGUGGAAUG	ID. NO. 37
53	GUGGAAUGAAGAGUC UUCCAUCCUACUGU	ID. NO. 38
55	GGAAUGAAGAGUCUU CCAUCCUACUGUUG	ID. NO. 39
56	GAAUGAAGAGUCUUC CAUCCUACUGUUGC	ID. NO. 40
61	GAAGAGUCUCCAUC CUACUGUUGCUGUGC	ID. NO. 41
64	GAGUCUCCAUCCUA CUGUUGCUGUGGUG	ID. NO. 42
69	UUCCAUCCUACUGUU GCUGUGGUGGCGAGU	ID. NO. 43
85	GCUGUGGUGGCGAGUU UGCUCAGCCUAUCCA	ID. NO. 44
86	CUGUGGUGGCGAGUUU GCUCAGCCUAUCCAU	ID. NO. 45
90	GCGUGGCGAGUUUGCUC AGCCUAUCCAUUGGA	ID. NO. 46
96	CAGUUUGCUCAGCCUA UCCAUUGGAUGGAGC	ID. NO. 47
98	GUUUGCUCAGCCUAC CAUUGGAUGGAGCUG	ID. NO. 48
102	GCUCAGCCUAUCCAUU GGAUGGAGCUGCAAG	ID. NO. 49
142	CACCAGCAUGAACCUU GUUCAGAAAUUCUA	ID. NO. 50
145	CAGCAUGAACCUUGUU CAGAAAUUCUAGAA	ID. NO. 51
146	AGCAUGAACCUUGUUC AGAAAUUCUAGAAA	ID. NO. 52
153	ACCUUGUUCAGAAUA UCUAGAAAACUACUA	ID. NO. 53
155	CUUGUUCAGAAUAUC UAGAAAACUACUACG	ID. NO. 54
157	UGUUCAGAAAUUCUA GAAAACUACUACGAC	ID. NO. 55
165	AAUAUCUAGAAAACUA CUACGACCUCAAAAA	ID. NO. 56
168	AUCUAGAAAACUACUA CGACCUCAAAAAGA	ID. NO. 57
175	AAACUACUACGACCUC AAAAAAGAUUGAGAA	ID. NO. 58
195	AAGAUUGAGAAACAGUU UGUUAGGAGAAAGGA	ID. NO. 59
196	AGAUUGAGAAACAGUUU GUUAGGAGAAAGGAC	ID. NO. 60

199	UGUGAAACAGUUUGUU AGGAGAAAGGACAGU	ID. NO. 61
200	GUGAAACAGUUUGUUA GGAGAAAGGACAGUG	ID. NO. 62
218	AGAAAGGACAGUGGUC CUGUUGUUAAAAAA	ID. NO. 63
223	GGACAGUGGUCCUGUU GUUAAAAAAUCCGA	ID. NO. 64
226	CAGUGGUCCUGUUGUU AAAAAAUCCGAGAA	ID. NO. 65
227	AGUGGUCCUGUUGUUA AAAAAAUCCGAGAA	ID. NO. 66
235	UGUUGUUAAAAAAUC CGAGAAUUCGAGAAG	ID. NO. 67
252	GAGAAUUCGAGAAGUU CCUUGGAIUGGAGGU	ID. NO. 68
253	AGAAUUCGAGAAGUUC CUUGGAIUGGAGGUG	ID. NO. 69
256	AAUUCGAGAAGUCCUU GGAIUGGAGGUGAAG	ID. NO. 70
261	AGAAGUUCUUGGAIU GGAGGUGACGGGGAA	ID. NO. 71
285	CGGGGAAGCUGGACUC CGACACUCUGGAGGU	ID. NO. 72
293	CUGGACUCCGACACUC UGGAGGUGAUGGCA	ID. NO. 73
325	GCCCAGGUGUGGAGUU CCUGAUGUUGGUCAC	ID. NO. 74
326	CCCAGGUGUGGAGUUC CUGAUGUUGGUCACU	ID. NO. 75
334	UGGAGUUCUGAUGUU GGUCACUUCAGAACC	ID. NO. 76
338	GUCCUGAUGUUGGUC ACUUCAGAACCUUC	ID. NO. 77
342	CUGAUGUUGGUCACUU CAGAACCUUCCUGG	ID. NO. 78
343	UGAUGUUGGUCACUUC AGAACCUUCCUGGC	ID. NO. 79
351	GUCACUUCAGAACCUU UCCUGGCAUCCCGAA	ID. NO. 80
352	UCACUUCAGAACCUUU CCUGGCAUCCCGAAG	ID. NO. 81
353	CACUUCAGAACCUUUC CUGGCAUCCCGAAGU	ID. NO. 82
361	AACCUUCCUGGCAUC CCGAAGUGGAGGAAA	ID. NO. 83
385	GAGGAAAACCCACCUU ACAUACAGGAUUGUG	ID. NO. 84
386	AGGAAAACCCACCUUA CAUACAGGAUUGUGA	ID. NO. 85
390	AAACCCACCUUACAU CAGGAUUGUGAAUUA	ID. NO. 86
397	CCUUAUACAGGAUU GUGAAUUAUACACCA	ID. NO. 87
404	UACAGGAUUGUGAAUU AUACACCAGAUUUGC	ID. NO. 88
405	ACAGGAUUGUGAAUUA UACACCAGAUUUGC	ID. NO. 89
407	AGGAUUGUGAAUUAU CACCAGAUUUGCCAA	ID. NO. 90
416	AAUUAUACACCAGAUU UGCCAAAAGAUGCUG	ID. NO. 91
417	AUUAUACACCAGAUU GCCAAAAGAUGCUGU	ID. NO. 92
433	GCCAAAAGAUGCUGUU GAUUCUGCUGUUGAG	ID. NO. 93
437	AAAGAUGCUGUUGAUU CUGCUGUUGAGAAAG	ID. NO. 94
438	AAGAUGCUGUUGAUUC UGCUGUUGAGAAAGC	ID. NO. 95
445	UGUUGAUUCUGCUGUU GAGAAAGCUCUGAAA	ID. NO. 96

455	GCUGUUGAGAAAGCUC	UGAAAGUCUGGGAAG	ID. NO. 97
463	GAAAGCUCUGAAAGUC	UGGGAAGAGGUGACU	ID. NO. 98
479	UGGGAAGAGGUGACUC	CACUCACAUUCUCCA	ID. NO. 99
484	AGAGGUGACUCCACUC	ACAUUCUCCAGGCUG	ID. NO. 100
489	UGACUCCACUCACAUU	CUCCAGGCUGUAUGA	ID. NO. 101
490	GACUCCACUCACAUUC	UCCAGGCUGUAUGAA	ID. NO. 102
492	CUCCACUCACAUUCUC	CAGGCUGUAUGAAGG	ID. NO. 103
501	CAUUCUCCAGGCUGUA	UGAAGGAGAGGCUGA	ID. NO. 104
518	GAAGGAGAGGCUGAUA	UAUGAUCUCUUUUG	ID. NO. 105
520	AGGAGAGGCUGAUAUA	AUGAUCUCUUUUGCA	ID. NO. 106
526	GGCUGAUAUAUGAUC	UCUUUUGCAGUAGA	ID. NO. 107
528	CUGAUAUAUGAUCUC	UUUUGCAGUAGAGA	ID. NO. 108
530	GAUAUAUGAUCUCUU	UUGCAGUAGAGAAC	ID. NO. 109
531	AUUAUAUGAUCUCUUU	UGCAGUAGAGAACAA	ID. NO. 110
532	UAUAUAUGAUCUCUUU	GCAGUAGAGAACAU	ID. NO. 111
538	GAUCUCUUUUGCAGUU	AGAGAACAUGGAGAC	ID. NO. 112
539	AUCUCUUUUGCAGUUA	GAGAACAUGGAGACU	ID. NO. 113
555	GAGAACAUGGAGACUU	UUAOCUUUUGAUGG	ID. NO. 114
556	AGAACAUGGAGACUUU	UAOCUUUUGAUGGA	ID. NO. 115
557	GAACAUGGAGACUUUU	ACOCUUUUGAUGGAC	ID. NO. 116
558	AACAUGGAGACUUUA	CCCUUUUGAUGGACC	ID. NO. 117
563	GGAGACUUUUAOCUUU	UUGAUGGACCUGGAA	ID. NO. 118
564	GAGACUUUUAOCUUU	UGAUGGACCUGGAAA	ID. NO. 119
565	AGACUUUUAOCUUUU	GAUGGACCUGGAAAU	ID. NO. 120
583	UGGACCUGGAAAUUUU	UUGGCCCAUGCCUAU	ID. NO. 121
584	GGACCUGGAAAUUUU	UGGCCCAUGCCUAUG	ID. NO. 122
585	GACCUGGAAAUUUUU	GGCCCAUGCCUAUGC	ID. NO. 123
597	UUUUGGCCCAUGCCUA	UGCCCUUGGGOCAGG	ID. NO. 124
616	CCCUUGGCCCAGGGAUU	AAUGGAGAUGCCAC	ID. NO. 125
617	CCUGGCCCAGGGAUUA	AUGGAGAUGCCACU	ID. NO. 126
633	AUGGAGAUGCCACUU	UGAUGAUGAUGAACA	ID. NO. 127
634	UGGAGAUGCCACUUU	GAUGAUGAUGAACAA	ID. NO. 128
662	CAUGGACAAAGGALIA	CAACAGGGACCAAUU	ID. NO. 129
677	ACAACAGGGACCAAUU	UAUUUCUGUUGCUG	ID. NO. 130
678	CAACAGGGACCAAUUU	AUUUCUGUUGCUGC	ID. NO. 131
679	AACAGGGACCAAUUUA	UUUCUGUUGCUGCU	ID. NO. 132

681	CAGGGACCAAUUUAU UCUUGUUGCUGCUCA	ID. NO. 133
682	AGGGACCAAUUUAUU CUUGUUGCUGCUCAU	ID. NO. 134
683	GGGACCAAUUUAUUUC UCGUUGCUGCUCAUG	ID. NO. 135
685	GACCAAUUUAUUUCUC GUUGCUGCUCAUGAA	ID. NO. 136
688	CAAUUUAUUUCUUGU GCUGCUCAUGAAAUU	ID. NO. 137
695	UUUCUUGUUGCUGCUC AUGAAAUUGGCCACU	ID. NO. 138
703	UGCUGCUCAUGAAAUU GGCCACUCCUGGGU	ID. NO. 139
711	AUGAAAUUGGCCACUC CCUGGGUCUCUUUCA	ID. NO. 140
719	GGCCACUCCUGGGUC UCUUUCACUCAGCCA	ID. NO. 141
721	CCACUCCUGGGUCUC UUUCACUCAGCCAAC	ID. NO. 142
723	ACUCCUGGGUCUCU UCACUCAGCCAACAC	ID. NO. 143
724	CUCCUGGGUCUCUU CACUCAGCCAACACU	ID. NO. 144
725	UCCUGGGUCUCUUC ACUCAGCCAACACUG	ID. NO. 145
729	UGGGUCUCUUUCACUC AGCCAACACUGAAGC	ID. NO. 146
746	GCCAACACUGAAGCUU UGAUGUACCCACUCU	ID. NO. 147
747	CCAACACUGAAGCUU GAUGUACCCACUCUA	ID. NO. 148
753	CUGAAGCUUGAUGUA CCCACUCUAUCACUC	ID. NO. 149
760	UUUGAUGUACCCACUC UAUCACUCACUCACA	ID. NO. 150
762	UGAUGUACCCACUCUA UCACUCACUCACAGA	ID. NO. 151
764	AUGUACCCACUCUAUC ACUCACUCACAGACC	ID. NO. 152
768	ACCCACUCUAUCACUC ACUCACAGACCUGAC	ID. NO. 153
772	ACUCUAUCACUCACUC ACAGACCUGACUCGG	ID. NO. 154
785	CUCACAGACCUGACUC GGUUCCGCCUGUCUC	ID. NO. 155
789	CAGACCUGACUCGGU CCGCUGUCUCAAGA	ID. NO. 156
790	AGACCUGACUCGGUUC CGCCUGUCUCAAGAU	ID. NO. 157
798	CUCCGUUCCGCCUGUC UCAAGAUGAUUAAA	ID. NO. 158
800	CGGUUCCGCCUGUC AAGAUGAUUAAAUG	ID. NO. 159
809	CUGUCUCAAGAUGAUA UAAUUGGCAUUCAGU	ID. NO. 160
811	GUCUCAAGAUGAUUA AAUUGGCAUUCAGUCC	ID. NO. 161
820	UGAUUAAAUGGCAUU CAGUCCCUUAUGGA	ID. NO. 162
821	GAAUAAAUGGCAUUC AGUCCCUUAUGGAC	ID. NO. 163
825	UAAUUGGCAUUCAGUC CCUCUAUGGACCUCC	ID. NO. 164
829	UGGCAUUCAGUCCUC UAUGGACCUCCCCU	ID. NO. 165
831	GCAUUCAGUCCUCUA UGGACCUCCCCUGA	ID. NO. 166
839	UCCUCUAUGGACCUC CCCCUGACUCCCCUG	ID. NO. 167
849	GACCUCCCCCUGACUC CCCUGAGACCCCCU	ID. NO. 168

868	UGAGACCCCCUGGUA	CCCAAGGAACCUGUC	ID. NO. 169
883	ACCCACAGGAACCUGUC	CCUCCAGAACCCUGG	ID. NO. 170
887	ACGGAACCUGUCCUC	CAGAACCUGGGAGC	ID. NO. 171
917	CCAGCCAACUGUGAUC	CUGCUUUGUCCUUUG	ID. NO. 172
923	AACUGUGAUCCUGCUU	UGUCCUUUGAUGCUG	ID. NO. 173
924	ACUGUGAUCCUGCUUU	GUCCUUUGAUGCUGU	ID. NO. 174
927	GUGAUCCUGCUUUGUC	CUUUGAUGCUGUCAG	ID. NO. 175
930	AUCCUGCUUUGUCCUU	UGAUGCUGUCAGCAC	ID. NO. 176
931	UCCUGCUUUGUCCUUU	GAUGCUGUCAGCACU	ID. NO. 177
940	GUCCUUUGAUGCUGUC	AGCACUCUGAGGGGA	ID. NO. 178
947	GAUGCUGUCAGCACUC	UGAGGGGAGAAUCC	ID. NO. 179
961	UCUGAGGGGAGAAUUC	CUGAUCUUUAAAGAC	ID. NO. 180
967	GGGAGAAUCCUGAUC	UUUAAAGACAGGCAC	ID. NO. 181
969	GAGAAUCCUGAUCUU	UAAAGACAGGCACUU	ID. NO. 182
970	AGAAAUCCUGAUCUUU	AAAGACAGGCACUUU	ID. NO. 183
971	GAAAUCCUGAUCUUUA	AAGACAGGCACUUUU	ID. NO. 184
984	UUAAAGACAGGCACUU	UUGGCGCAAUCCCU	ID. NO. 185
985	UAAAGACAGGCACUUU	UGGCGCAAUCCUC	ID. NO. 186
986	AAAGACAGGCACUUUU	GGGCGCAAUCCUCA	ID. NO. 187
996	ACUUUUGGCGCAAUUC	CCUCAGGAAGCUUGA	ID. NO. 188
1000	UUGGCGCAAUCCUC	AGGAAGCUUGAACCU	ID. NO. 189
1009	AUCCUCAGGAAGCUU	GAACCUGAAUUGCAU	ID. NO. 190
1020	AGCUUGAACCUGAAUU	GCAUUUGAUCUCUUC	ID. NO. 191
1025	GAACCUGAAUUGCAUU	UGAUCUCUUCAUUUU	ID. NO. 192
1026	AACCUGAAUUGCAUUU	GAUCUCUUCAUUUUG	ID. NO. 193
1030	UGAAUUGCAUUUGAUC	UCUUCAUUUUGGCA	ID. NO. 194
1032	AAUUGCAUUUGAUCUC	UUCAUUUUGGCAUC	ID. NO. 195
1034	UUGCAUUUGAUCUCUU	CAUUUUGGCAUCUC	ID. NO. 196
1035	UGCAUUUGAUCUCUUC	AUUUUGGCAUCUCU	ID. NO. 197
1038	AUUUGAUCUCUUCAUU	UUGGCAUCUCUUC	ID. NO. 198
1039	UUUGAUCUCUUCAUUU	UGGCAUCUCUUCU	ID. NO. 199
1040	UUGAUCUCUUCAUUUU	GGCAUCUCUUCU	ID. NO. 200
1047	CUUCAUUUUGGCAUC	UCUCCUUCAGGCGU	ID. NO. 201
1049	UCAUUUUGGCAUCUC	UUCUUCAGGCGUGG	ID. NO. 202
1051	AUUUUGGCAUCUCUU	CCUUCAGGCGUGGAU	ID. NO. 203
1052	UUUUGGCAUCUCUUC	CUUCAGGCGUGGAUG	ID. NO. 204

1055	UGGCCAUCUCUCCU CAGGCGUGGAUGCG	ID. NO. 205
1056	GGCCAUCUCUCCUUC AGGCGUGGAUGCGC	ID. NO. 206
1074	GCGUGGAUGCGCAUA UGAAGUUAUAAGCAA	ID. NO. 207
1081	UGCGCAUAUGAAGUU ACUAGCAAGGACCUC	ID. NO. 208
1082	GCGCAUAUGAAGUUA CUAGCAAGGACCUCG	ID. NO. 209
1085	GCAUAUGAAGUUAUA GCAAGGACCUCGUU	ID. NO. 210
1096	UACUAGCAAGGACCUC GUUUUCAUUUUAAA	ID. NO. 211
1099	UAGCAAGGACCUCGUU UUCAUUUUAAAAGGA	ID. NO. 212
1100	AGCAAGGACCUCGUUU UCAUUUUAAAAGGAA	ID. NO. 213
1101	GCAAGGACCUCGUUUU CAUUUUAAAAGGAAA	ID. NO. 214
1102	CAAGGACCUCGUUUUC AUUUUUAAAAGGAAU	ID. NO. 215
1105	GGACCUCGUUUUCAU UUUAAAAGGAAAUCAA	ID. NO. 216
1106	GACCUCGUUUUCAUUU UUAAGGAAAUCAAU	ID. NO. 217
1107	ACCUCGUUUUCAUUUU UAAAGGAAAUCAAU	ID. NO. 218
1108	CCUCGUUUUCAUUUUU AAAGGAAAUCAAUC	ID. NO. 219
1109	CUCGUUUUCAUUUUUA AAGGAAAUCAAUCU	ID. NO. 220
1118	AUUUUUAAAAGGAAUC AAUUCUGGGCCAUA	ID. NO. 221
1122	UUAAGGAAAUCAAU CUGGGCCAUCAGAGG	ID. NO. 222
1123	UAAAGGAAAUCAAUC UGGGCCAUCAGAGGA	ID. NO. 223
1132	UCAAUUCUGGGCCAU CAGAGGAAAUAGAGGUA	ID. NO. 224
1147	CAGAGGAAAUAGAGGUA CGAGCUGGAAUACCA	ID. NO. 225
1158	AGGUACGAGCUGGAAU CCAAGAGGCAUCCA	ID. NO. 226
1171	AUACCCAAGAGGCAUC CACACCCUAGGUUC	ID. NO. 227
1180	AGGCAUCCACACCCUA GGUUCCCUCCAACC	ID. NO. 228
1184	AUCCACACCCUAGGUU UCCCUCCAACCGUGA	ID. NO. 229
1185	UCCACACCCUAGGUUU CCCUCCAACCGUGAG	ID. NO. 230
1186	CCACACCCUAGGUUC CCUCCAACCGUGAGG	ID. NO. 231
1190	AACCUAGGUUCCCUUC CAACCGUGAGGAAAA	ID. NO. 232
1207	AACCGUGAGGAAAUC GAUGCAGCCAUUUCU	ID. NO. 233
1219	AAUCGAUGCAGCCAUU UCUGAUAAGGAAAAG	ID. NO. 234
1220	AUCGAUGCAGCCAUUU CUGAUAAGGAAAAGA	ID. NO. 235
1221	UCGAUGCAGCCAUUUC UGAUAAGGAAAAGAA	ID. NO. 236
1226	GCAGCCAUUUCUGAUA AGGAAAAGAACAAAA	ID. NO. 237
1245	AAAAGAACAAAACAUU UUUCUUUGUAGAGGA	ID. NO. 238
1247	AAGAACAAAACAUUUU UCUUUGUAGAGGACA	ID. NO. 239
1248	AGAACAAAACAUUUU CUUUGUAGAGGACAA	ID. NO. 240

1249	GAACAAAACAUUUUC	UUUGUAGAGGACAAA	ID. NO. 241
1251	ACAAAACAUUUUCUU	UGUAGAGGACAAAU	ID. NO. 242
1252	CAAAACAUUUUCUUU	GUAGAGGACAAAUAC	ID. NO. 243
1255	AACAUUUUCUUUGUA	GAGGACAAAUACUGG	ID. NO. 244
1266	UUUGUAGAGGACAAUA	CUGGAGAUUUGAUGA	ID. NO. 245
1275	ACAAAUACUGGAGAUU	UGAUGAGAAGAGAAA	ID. NO. 246
1276	CAAUAUACUGGAGAUU	GAUGAGAAGAGAAAU	ID. NO. 247
1292	GAUGAGAAGAGAAAUU	CCAUGGAGCCAGGCU	ID. NO. 248
1293	AUGAGAAGAGAAAUUC	CAUGGAGCCAGGCUU	ID. NO. 249
1308	CCAUGGAGCCAGGCUU	UCCCAAGCAAUAAGC	ID. NO. 250
1309	CAUGGAGCCAGGCUUU	CCCAAGCAAUAAGCU	ID. NO. 251
1310	AUGGAGCCAGGCUUUC	CCAAGCAAUAAGCUG	ID. NO. 252
1321	CUUUCCAAGCAAUA	GCUGAAGACUUUCCA	ID. NO. 253
1332	AAUAAGCUGAAGACUU	UCCAGGGAUUGACUC	ID. NO. 254
1333	AUAAGCUGAAGACUUU	CCAGGGAUUGACUCA	ID. NO. 255
1334	AUAAGCUGAAGACUUUC	CAGGGAUUGACUCAA	ID. NO. 256
1342	AGACUUCCAGGGAUU	GACUCAAGAUUGAU	ID. NO. 257
1347	UCCAGGGAUUGACUC	AAAGAUUGAUGCUGU	ID. NO. 258
1354	GAUUGACUCAAGAUU	GAUGCUGUUUUGAA	ID. NO. 259
1363	AAAGAUUGAUGCUGUU	UUUGAAGAAUUUGGG	ID. NO. 260
1364	AAGAUUGAUGCUGUUU	UUGAAGAAUUUGGGU	ID. NO. 261
1365	AGAUUGAUGCUGUUUU	UGAAGAAUUUGGGUU	ID. NO. 262
1366	GAUUGAUGCUGUUUUU	GAAGAAUUUGGGUUC	ID. NO. 263
1374	CUGUUUUGAAGAAUU	UGGGUUCUUUAUUU	ID. NO. 264
1375	UGUUUUGAAGAAUUU	GGGUUCUUUAUUUC	ID. NO. 265
1380	UUGAAGAAUUUGGGUU	CUUUUAUUUCUUAC	ID. NO. 266
1381	UGAAGAAUUUGGGUUC	UUUUAUUUCUUUACU	ID. NO. 267
1383	AAGAAUUUGGGUUCUU	UUUAUUUCUUUACUGG	ID. NO. 268
1384	AGAAUUUGGGUUCUUU	UAUUUCUUUACUGGA	ID. NO. 269
1385	GAAUUUGGGUUCUUUU	AUUUCUUUACUGGAU	ID. NO. 270
1386	AAUUUGGGUUCUUUUA	UUUCUUUACUGGAUC	ID. NO. 271
1388	UUUGGGUUCUUUUAUU	UCUUUACUGGAUCUU	ID. NO. 272
1389	UUGGGUUCUUUUAUUU	CUUUACUGGAUCUUC	ID. NO. 273
1390	UGGGUUCUUUUAUUUC	UUUACUGGAUCUUCA	ID. NO. 274
1392	GGUUCUUUUAUUUCUU	UACUGGAUCUUCACA	ID. NO. 275
1393	GUUCUUUUAUUUCUUU	ACUGGAUCUUCACAG	ID. NO. 276

1394	UUCUUUUUAUUUCUUUA CUGGAUCUUCACAGU	ID. NO. 277
1401	AUUUCUUUACUGGAUC UUCACAGUUGGAGUU	ID. NO. 278
1403	UUCUUUACUGGAUCUU CACAGUUGGAGUUUG	ID. NO. 279
1404	UCUUUACUGGAUCUUC ACAGUUGGAGUUUGA	ID. NO. 280
1410	CUGGAUCUUCACAGUU GGAGUUUGAOCCTAA	ID. NO. 281
1416	CUUCACAGUUGGAGUU UGAOCCTAAUGCTAA	ID. NO. 282
1417	UUCACAGUUGGAGUUU GACCTAAUGCTAAG	ID. NO. 283
1448	AAAGUGACACACACUU UGAAGAGUACAGCU	ID. NO. 284
1449	AAGUGACACACACUUU GAAGAGUACAGCUG	ID. NO. 285
1457	CACACUUUGAAGAGUA ACAGCUGGCUUAAUU	ID. NO. 286
1468	GAGUACAGCUGGCUU AAUUGUUGAAAGAGA	ID. NO. 287
1469	AGUACAGCUGGCUUA AUUGUUGAAAGAGAU	ID. NO. 288
1472	AACAGCUGGCUUAAUU GUUGAAAGAGAUUG	ID. NO. 289
1475	AGCUGGCUUAAUUGUU GAAAGAGAUUGUAG	ID. NO. 290
1485	AUUGUUGAAAGAGAU UGUAGAAGGCACAAU	ID. NO. 291
1489	UUGAAAGAGAUUGUA GAAGGCACAUAUUG	ID. NO. 292
1501	UGUAGAAGGCACAUA UGGGCACUUUAAUG	ID. NO. 293
1510	CACAUAUUGGGCACUU UAAUGAAGCUAUA	ID. NO. 294
1511	ACAUAUUGGGCACUUU AAUGAAGCUAUAUA	ID. NO. 295
1512	CAUAUUGGGCACUUUA AAUGAAGCUAUAUAU	ID. NO. 296
1522	ACUUUAAUGAAGCUA AUAAUUCUUCACCUA	ID. NO. 297
1525	UUAAUGAAGCUAUA AUUCUUCACCUAAGU	ID. NO. 298
1528	AAUGAAGCUAUAUAU CUUCACCUAAGUCUC	ID. NO. 299
1529	AUGAAGCUAUAUAUC UUCACCUAAGUCUCU	ID. NO. 300
1531	GAAGCUAUAUAUUCU CACCUAAGUCUCUGU	ID. NO. 301
1532	AAGCUAUAUAUUCUC ACCUAAGUCUCUGUG	ID. NO. 302
1537	AAUAUAUUCUUCACCUA AGUCUCUGUGAAUUG	ID. NO. 303
1541	AUUCUUCACCUAAGUC UCUGUGAAUUGAAAU	ID. NO. 304
1543	UCUUCACCUAAGUCUC UGUGAAUUGAAUGU	ID. NO. 305
1551	UAAGUCUCUGUGAAUU GAAUGUUCGUUUUC	ID. NO. 306
1559	UGUGAAUUGAAUGUU CGUUUUCUCCUGCCU	ID. NO. 307
1560	GUGAAUUGAAUGUUC GUUUUUCUCCUGCCUG	ID. NO. 308
1563	AAUUGAAUGUUCGUU UUCUCCUGCCUGUGC	ID. NO. 309
1564	AUUGAAUGUUCGUUU UCUCUGCCUGUGCU	ID. NO. 310
1565	UUGAAUGUUCGUUUU CUCCUGCCUGUGCUG	ID. NO. 311
1566	UGAAUGUUCGUUUUC UCCUGCCUGUGCUGU	ID. NO. 312

1568	AAAUUUUUUUUUUCUC	CUGOCUGUGCUGUGA	ID. NO. 313
1586	GCCUGUGCUGUGACUC	GAGUCACACUCAAGG	ID. NO. 314
1591	UGCUGUGACUUGAGUC	ACACUCAAGGGAACU	ID. NO. 315
1597	GACUUGAGUCACACUC	AAGGGAACUUGAGOG	ID. NO. 316
1607	ACACUCAAGGGAACUU	GAGOGUGAUCUGUA	ID. NO. 317
1618	AACUUGAGOGUGAAUC	UGUAUCUUGOOGGUC	ID. NO. 318
1622	UGAGOGUGAUCUGUA	UCUUGOOGGUCAUUU	ID. NO. 319
1624	AGCGUGAUCUGUAUC	UUGOOGGUCAUUUUU	ID. NO. 320
1626	CGUGAUCUGUAUCUU	GCOGGUCAUUUUUUAU	ID. NO. 321
1633	CUGUAUCUUGOOGGUC	AUUUUUAUGUAUAUA	ID. NO. 322
1636	UAUCUUGOOGGUCAUU	UUUAUGUAUAUAACAG	ID. NO. 323
1637	AUCUUGOOGGUCAUUU	UUAUGUAUAUAACAGG	ID. NO. 324
1638	UCUUGOOGGUCAUUUU	UAUGUAUAUAACAGGG	ID. NO. 325
1639	CUUGOOGGUCAUUUUU	AUGUAUAUAACAGGGC	ID. NO. 326
1640	UUGOOGGUCAUUUUUUA	UGUAUAUAACAGGGCA	ID. NO. 327
1644	CGGUCAUUUUUUAUGUU	AUAACAGGGCAUUA	ID. NO. 328
1645	GGUCAUUUUUUAUGUUA	UAACAGGGCAUUCAA	ID. NO. 329
1647	UCAUUUUUAUGUUAUU	ACAGGGCAUUCAAAU	ID. NO. 330
1648	CAUUUUUAUGUUAUAUA	CAGGGCAUUCAA AUG	ID. NO. 331
1657	GUUAUAACAGGGCAUU	CAAUUGGGCUGCUGC	ID. NO. 332
1658	UUAUAUAACAGGGCAUUC	AAAUUGGGCUGCUGCU	ID. NO. 333
1674	AAAUUGGGCUGCUGCUU	AGCUUGCACCUUGUC	ID. NO. 334
1675	AAUUGGGCUGCUGCUUA	GCUUGCACCUUGUCA	ID. NO. 335
1679	GGCUGCUGCUUAGCUU	GCACCUUGUCACAUUA	ID. NO. 336
1686	GCUUAGCUUGCACCUU	GUCACAUAGAGUGAU	ID. NO. 337
1689	UAGCUUGCACCUUGUC	ACAUAGAGUGAUCUU	ID. NO. 338
1694	UGCACCUUGUCACAUUA	GAGUGAUCUUUCCCA	ID. NO. 339
1702	GUCACAUAGAGUGAUC	UUUCCCAAGAGAAGG	ID. NO. 340
1704	CACAUAGAGUGAUCUU	UCCCAAGAGAAGGGG	ID. NO. 341
1705	ACAUAGAGUGAUCUUU	CCCAAGAGAAGGGGA	ID. NO. 342
1706	CAUAAGAGUGAUCUUUC	CCAAGAGAAGGGGAA	ID. NO. 343
1727	AGAAGGGGAAGCACUC	GUGUGCAACAGACAA	ID. NO. 344
1751	CAGACAAGUGACUGUA	UCUGUGUAGACUAUU	ID. NO. 345
1753	GACAAGUGACUGUAUC	UGUGUAGACUAUUUG	ID. NO. 346
1759	UGACUGUAUCUGUGUA	GACUAUUUGCUUAUU	ID. NO. 347
1764	GUACUGUGUAGACUA	UUUGCUUAUUUAUA	ID. NO. 348

1766	AUCUGUGUAGACUAAU UGCUAAUUUAAUAAA	ID. NO. 349
1767	UCUGUGUAGACUAAUU GCUAAUUUAAUAAAG	ID. NO. 350
1771	UGUAGACUAAUUGCUU AUUUAUAAGACGA	ID. NO. 351
1772	GUAGACUAAUUGCUUA UUAUAUAAAGACGAU	ID. NO. 352
1774	AGACUAAUUGCUAAUU UAUAUAAAGACGAUU	ID. NO. 353
1775	GACUAAUUGCUAAUU AAUAUAAAGACGAUUG	ID. NO. 354
1776	ACUAAUUGCUAAUUUA AUAAAGACGAUUGU	ID. NO. 355
1779	AUUUGCUAAUUUAUA AAGACGAUUUGUCAG	ID. NO. 356
1788	UUUAUAUAAAGACGAUU UGUCAGUUGUUUU	ID. NO. 357
1789	UUAUAUAAAGACGAUUU GUCAGUUGUUUU	ID. NO. 358
1792	AUAAGACGAUUUGUC AGUUGUUUU	ID. NO. 359

Table AIV: Human Stromelysin HP Target Sequence

nt.		
Position	Target Sequence	Seq. ID. NO.
66	CUACU GUU GCUGUGCGUGGCAGU	ID. NO. 360
82	UGGCA GUU UGCUCAGCCUAUCCA	ID. NO. 361
192	AAACA GUU UGUUAGGAGAAAGGA	ID. NO. 362
430	AUGCU GUU GAUUCUGCGUGAG	ID. NO. 363
442	CUGCU GUU GAGAAAGCUCUGAAA	ID. NO. 364
775	UCACA GAC CUGACUCCGUUCCGC	ID. NO. 365
1360	AUGCU GUU UUUGAAGAAUUUGGG	ID. NO. 366
1407	UCACA GUU GGAGUUUGACCCAAA	ID. NO. 367

Table AV: Human HH Ribozyme Sequence

nt. Position.	Ribozyme Sequence	Seq. ID.
10	GUUGUCUC CUGAAGAGCACGAAAGUGCGAA AUGCCUUG	ID.NO.375
21	UUAGCUC CUGAUGAGGCCGAAAGGCCGAA AUGUUGU	ID.NO.376
168	GAGGUCG CUGAUGAGGCCGAAAGGCCGAA AGUAGUU	ID.NO.377
616	CUCCAUU CUGAUGAGGCCGAAAGGCCGAA AUCCCTUG	ID.NO.378
617	UCUCCAU CUGAUGAGGCCGAAAGGCCGAA AAUCCCU	ID.NO.379
633	CAUCAUCA CUGAAGAGCACGAAAGUGCGAA AGUGGGCA	ID.NO.380
634	UCAUCAUC CUGAAGAGCACGAAAGUGCGAA AAGUGGGC	ID.NO.381
662	CCUGUUG CUGAUGAGGCCGAAAGGCCGAA AUCCUUU	ID.NO.382
711	ACCCAGG CUGAUGAGGCCGAAAGGCCGAA AGUGGGC	ID.NO.383
820	GGGACUG CUGAUGAGGCCGAAAGGCCGAA AUGCCAU	ID.NO.384
883	UCUGGAGG CUGAAGAGCACGAAAGUGCGAA ACAGGUUC	ID.NO.385
947	CCCCUCA CUGAUGAGGCCGAAAGGCCGAA AGUGCUG	ID.NO.386
996	CCUGAGG CUGAUGAGGCCGAAAGGCCGAA AUUUGCG	ID.NO.387
1123	UGGCCCA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU	ID.NO.388
1132	UUUCCUCU CUGAUGAGCACGAAAGUGCGAA AUGGCCCA	ID.NO.389
1221	CCUUAUCA CUGAAGAGCACGAAAGUGCGAA AAAUGGCU	ID.NO.390
1266	UCUCCAG CUGAUGAGGCCGAAAGGCCGAA AUUUGUC	ID.NO.391
1275	UCUCAUCA CUGAAGAGCACGAAAGUGCGAA AUCUCCAG	ID.NO.392
1334	AUCCCTUG CUGAUGAGGCCGAAAGGCCGAA AAAGUCU	ID.NO.393
1354	CAGCAUC CUGAUGAGGCCGAAAGGCCGAA AUCUUUG	ID.NO.394
1363	UCUUCAAA CUGAUGAGCACGAAAGUGCGAA ACAGCAUC	ID.NO.395
1410	AAACUCC CUGAUGAGGCCGAAAGGCCGAA ACUGUGA	ID.NO.396

Table AVI: Rabbit Stromelysin HH Ribozyme Target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
18	CAAGGCAU C AAGACAGC	345	CCUGAUGU U GGUCACUU
29	GACAGCAU A GAGCUGAG	349	AUGUUGGU C ACUUCAGU
39	AGCUGAGU A AAGCCAAU	353	UGGUCACU U CAGUACCU
61	UGAAAACU C UUCCAACC	354	GGUCACUU C AGUACCUU
63	AAAACUCU U CCAACCCU	358	ACUUCAGU A CCUUCCCU
64	AAACUCUU C CAACCCUG	362	CAGUACCU U CCCUGGCA
75	ACCCUGCU A CUGCUGUG	363	AGUACCUU C CCUGGCAC
93	GUGGCGCU U UGCUCAGC	391	CAAAAACU C ACCUAACU
94	UGGCGCUU U GCUCAGCC	396	ACUCACCU A ACUACAG
98	GCUUUGCU C AGCCUAUC	400	ACCUAACU U ACAGGAUU
104	CUCAGCCU A UCCACUGG	401	CCUAACUU A CAGGAUUG
106	CAGCCUAU C CACUGGAU	408	UACAGGAU U GUGAAUUA
122	UGGAGCCU C AAGGGAUG	415	UUGUGAAU U ACACACCG
153	AUGGACCU U CUUCAGCA	416	UGUGAAUU A CACACCGG
154	UGGACCUU C UUCAGCAA	427	CACCGGAU C UGCCAAGA
156	GACCUUCU U CAGCAAUA	444	GAUGCUGU U GAUGCUGC
157	ACCUUCUU C AGCAAUAU	456	GCUGCCAU U GAGAAAGC
164	UCAGCAAU A UCUGGAAA	466	AGAAAGCU C UGAAGGUC
166	AGCAAUAU C UGGAAAAC	474	CUGAAGGU C UGGGAGGA
176	GGAAAACU A CUACAACC	490	AGGUGACU C CACUCACG
179	AAACUACU A CAACCUUG	495	ACUCCACU C ACGUUCUC
186	UACAACCU U GAAAAAGA	500	ACUCACGU U CUCCAGGA
206	GAAACAGU U UGUUAAAA	501	CUCACGUU C UCCAGGAA
207	AAACAGUU U GUUAAAAG	503	CACGUUCU C CAGGAAGU
210	CAGUUUGU U AAAAGAAA	512	CAGGAAGU A UGAAGGAG
211	AGUUUGUU A AAAGAAAG	531	GCUGACAU A AUGAUCUC
226	AGGACAGU A GUCCUGUU	537	AUAAUGAU C UCUUUUGG
229	ACAGUAGU C CUGUUGUU	539	AAUGAUCU C UUUUGGAG
234	AGUCCUGU U GUUAAAAA	541	UGAUCUCU U UUGGAGUC
237	CCUGUUGU U AAAAAAAU	542	GAUCUCUU U UGGAGUCC
238	CUGUUGUU A AAAAAAUC	543	AUCUCUUU U GGAGUCCG
246	AAAAAAAU C CAAGAAAU	549	UUUGGAGU C CGAGAACA
263	GCAGAAGU U CCUUGGCU	565	AUGGAGAU U UUAUCCCU
264	CAGAAGUU C CUUGGCUU	566	UGGAGAUU U UAUUCCUU
267	AAGUUCCU U GGCUUGGA	567	GGAGAUUU U AUUCCUUU
272	CCUUGGCU U GGAGGUGA	568	GAGAUUUU A UUCCUUUU
296	GCUGGACU C CAACACCC	570	GAUUUUUAU U CCUUUUGA
315	GAGGUGAU A CGCAAGCC	571	AUUUUUAU C CUUUUGAU
336	UGUGGCGU U CCUGAUGU	574	UUAUCCCU U UUGAUGGA
337	GUGGCGUU C CUGAUGUU	575	UAUUCCUU U UGAUGGAC

576	AUUCUUU U GAUGGACC	905	UCCAGGAU C UGGGACCC
594	GGAAUGU U UUGGCUCA	918	ACCCAGU C AUGUGUGA
595	GAAUGUU U UGGCUCAU	928	UGUGUGAU C CAGAUCUG
596	AAAUGUUU U GGCUCAUG	934	AUCCAGAU C UGUCCUUC
601	UUUUGGCU C AUGCUUUAU	938	AGAUCUGU C CUUCGAUG
607	CUCAUGCU U AUGCACC U	941	UCUGUCCU U CGAUGCAA
608	UCAUGCUU A UGCACCUG	942	CUGUCCU C GAUGCAAU
627	CCAGGAU U AAUGGAGA	951	GAUGCAAU C AGCACUCU
628	CAGGAUU A AUGGAGAU	958	UCAGCACU C UGAGGGGA
644	UGCCCACU U UGAUGAUG	972	GGAGAAU U CUGUUCU
645	GCCCACU U GAUGAUGA	973	GAGAAU C UGUUCUU
673	CAAAGGAU A CAACAGGA	977	AAUUCUGU U CUUUAAG
688	GAACCAU U UAUUCCU	978	AUUCUGU C UUUAAAGA
689	AACCAAU U AUUCCUUG	980	UCUGUUCU U UAAAGACA
690	ACCAUUU A UUCCUUGU	981	CUGUUCU U AAAGACAG
692	CAAUUUU U CCUUGUUG	982	UGUUCUU A AAGACAGG
693	AAUUUAU C CUUGUUGC	992	AGACAGGU A UUUCUGGC
696	UUAUCCU U GUUGCUGC	994	ACAGGUU U UCUGCGC
699	UUCUUGU U GCUGCUCA	995	CAGGUUU U CUGGCGCA
706	UUGCUGCU C AUGAGCUU	996	AGGUUUU C UGGCGCAA
714	CAUGAGCU U GGCCACUC	1007	GCGCAAGU C CCUCAGGA
722	UGGCCACU C CCUGGGUC	1011	AAGUCCU C AGGAUUCU
730	CCCUGGGU C UGUUUCAC	1017	CUCAGGAU U CUCGAACC
734	GGGUCUGU U UCACUCGG	1018	UCAGGAU C UCGAACCU
735	GGUCUGU U CACUCGGC	1020	AGGAUUCU C GAACCTUGA
736	GUCUGUU C ACUCGGCC	1031	ACCUGAGU U UCAUUUGA
740	GUUUCACU C GGCCAACC	1032	CCUGAGUU U CAUUUGAU
764	GCUGAUGU A CCCAGUCU	1033	CUGAGUU C AUUUGAUC
771	UACCCAGU C UACAACGC	1036	AGUUUCAU U UGAUCUCU
773	CCCAGUCU A CAACGCCU	1037	GUUUCAU U GAUCUCU
782	CAACGCCU U CACAGACC	1041	CAUUUGAU C UCUUCAU
783	AACGCCU C ACAGACCU	1043	UUUGAUCU C UUCAUUCU
800	GGCCCGGU U CCGCCUUU	1045	UGAUCUCU U CAUUCUGG
801	GCCCGGU C CGCCUUUC	1046	GAUCUCU C AUUCUGGC
807	UUCCGCCU U UCUCAAGA	1049	CUCUUCAU U CUGGCCAU
808	UCCGCCU U CUCAAGAU	1050	UCUUCAU C UGGCCAUC
809	CCGCCUU C UCAAGAUG	1058	CUGGCCAU C UCUCCU
811	GCCUUUCU C AAGAUGAU	1060	GGCCAUCU C UUCCUUA
831	GAUGGCAU C CAAUCCU	1062	CCAUCUCU U CCUUCAGC
836	CAUCCAU C CCUCUAUG	1063	CAUCUCU C CUUCAGCA
840	CAAUCCU C UAUGGACC	1066	CUCUCCU U CAGCAGUG
842	AUCCUCU A UGGACCGG	1067	UCUCCU C AGCAGUGG
860	CCUGCCU C UCCUGAUA	1085	UGCUGCAU A UGAAGUUA
862	CUGCCUCU C CUGAUAAC	1092	UAUGAAGU U AUUAGCAG
868	CUCCUGAU A ACUCUGGA	1093	AUGAAGU A UUAGCAGG
872	UGAUAACU C UGGAGUGC	1095	GAAGUUU U AGCAGGGA
883	GAGUGCCU A UGGAACCU	1096	AAGUUUU A GCAGGGAU
894	GAACCGU C CCUCCAGG	1105	GCAGGGAU A CUGUUUUC
898	CUGUCCU C CAGGAUCU	1110	GAUACUGU U UUCAUUU

1111	AUACUGUU U UCAUUUUU	1374	GAUGCUGU U UUUGAAGC
1112	UACUGUUU U CAUUUUUA	1375	AUGCUGUU U UUGAAGCA
1113	ACUGUUUU C AUUUUUAA	1376	UGCUGUUU U UGAAGCAU
1116	GUUUUCAU U UUUAAAGG	1377	GCUGUUUU U GAAGCAUU
1117	UUUUUCAU U UUAAGGGA	1385	UGAAGCAU U UGGGUUUU
1118	UUUCAUUU U UAAAGGAA	1386	GAAGCAUU U GGGUUUUU
1119	UUCAUUUU U AAAGGAAC	1391	AUUUGGGU U UUUCUAUU
1120	UCAUUUUU A AAGGAACU	1392	UUUGGGUU U UUCUAUUU
1129	AAGGAACU C AGUUCUGG	1393	UUGGGUUU U UCUAUUUC
1133	AACUCAGU U CUGGGCCA	1394	UGGGUUUU U CUUUUUUC
1134	ACUCAGUU C UGGGCCAU	1395	GGGUUUUU C UAUUUUCU
1143	UGGGCCAU U AGAGGAAA	1397	GUUUUUUC A UUUCUUA
1144	GGGCCAUU A GAGGAAAU	1399	UUUUCUAU U UCUUCAGU
1158	AAUGAGGU A CAAGCUGG	1400	UUUCUAUU U CUUCAGUG
1168	AAGCUGGU U ACCCAAGA	1401	UUCUAUUU C UUCAGUGG
1169	AGCUGGUU A CCAAGAA	1403	CUAUUUUC U CAGUGGAU
1182	AGAAGCAU C CACACCCU	1404	UAUUUCUU C AGUGGAUC
1195	CCUGGGGU U UCCCUUCA	1412	CAGUGGAU C UUCACAGU
1196	CCUGGGUU U CCCUCAA	1414	GUGGAUCU U CACAGUCG
1197	CUGGGUUU C CCUUAAC	1415	UGGAUCUU C ACAGUCGG
1201	GUUUCCCU U CAACCAUA	1421	UUCACAGU C GGAGUUUG
1202	UUUCCCUU C AACCAUAA	1427	GUCGGAGU U UGACCCAA
1209	UCAACCAU A AGAAAAAU	1428	UCGGAGUU U GACCCAAA
1218	AGAAAAAU U GAUGCUGC	1458	ACACAUGU U UUGAAGAG
1230	GCUGCCAU U UCUGAUAA	1459	CACAUGUU U UGAAGAGC
1231	CUGCCAUU U CUGAUAAAG	1460	ACAUGUUU U GAAGAGCA
1232	UGCCAUUU C UGAUAAGG	1478	CAGCUGGU U UCAGUGUU
1237	UUUCUGAU A AGGAAAGG	1479	AGCUGGUU U CAGUGUUA
1256	GAAAACAU A CUUCUUUG	1480	GCUGGUUU C AGUGUUAG
1259	AACAUACU U CUUUGUGG	1486	UUCAGUGU U AGGAGGGG
1260	ACAUACUU C UUUGUGGA	1487	UCAGUGUU A GGAGGGGU
1262	AUACUUCU U UGUGGAAG	1498	AGGGGUGU A UAGAAGGC
1263	UACUUCUU U GUGGAAGA	1500	GGGUGUAA A GAAGGCAC
1277	AGACAAAU A CUGGAGGU	1519	AUGAAUGU U UUAUAUGA
1286	CUGGAGGU U UGAUGAGA	1520	UGAAUGUU U UAAUUGAA
1287	UGGAGGUU U GAUGAGAA	1521	GAAUGUUU U AAAUGAAC
1304	GAGACAGU C CCUGGAGC	1522	AAUGUUUU A AAUGAACC
1319	GCCAGGCU U UCCCAGAC	1532	AUGAACCU A AUUGUACA
1320	CCAGGCUU U CCCAGACA	1535	AACCUAAU U GUUCAACA
1321	CAGGCUUU C CCAGACAU	1538	CUAAUUGU U CAACACTU
1330	CCAGACAU A UAGCAGAA	1539	UAAUUGUU C AACACTUA
1332	AGACAUAA A GCAGAAGA	1546	UCAACACU U AGGACTUU
1343	AGAAGACU U UCCAGGAA	1547	CAACACUU A GGACTUUG
1344	GAAGACUU U CCAGGAAU	1553	UUAGGACU U UGUGAGUU
1345	AAGACUUU C CAGGAAUU	1554	UAGGACUU U GUGAGUUG
1353	CCAGGAAU U AAUCCAAA	1561	UUGUGAGU U GAAGUGGC
1354	CAGGAAUU A AUCCAAAG	1571	AAGUGGCU C AUUUUCUC
1357	GAAUUAUU C CAAAGAUC	1574	UGGCUCAU U UUCUCCUG
1365	CCAAAGAU C GAUGCUGU	1575	GGCUCAUU U UCUCUCCG

1576	GCUCAUUU	U	CUCCUGCA
1577	CUCAUUUU	C	UCCUGCAU
1579	CAUUUUUCU	C	CUGCAUAU
1586	UCCUGCAU	A	UGCUGUGA
1602	AUGGGAAU	C	UCGAGCAU
1604	GGGAAUCU	C	GAGCAUGA
1620	AACUGUGU	A	UCUAAACUG
1622	CUGUGUAU	C	UAACUGGA
1624	GUGUAUCU	A	ACUGGACU
1633	ACUGGACU	U	UGCACAUC
1634	CUGGACUU	U	GCACAUCG
1641	UUGCACAU	C	GUUACGGG
1644	CACAUCGU	U	ACGGGUGU
1645	ACAUCGUU	A	CGGGUGUU
1653	ACGGGUGU	U	CAAACAGG
1654	CGGGUGUU	C	AAACAGGC
1670	CUGCUGCU	U	AGCUUGCA
1671	UGCUGCUU	A	GCUUGCAC
1675	GCUUAGCU	U	GCACUUGA
1681	CUUGCACU	U	GAUCACAU
1685	CACUUGAU	C	ACAUGGAA
1701	AGGGAGCU	U	CCACGAGA
1702	GGGAGCUU	C	CACGAGAC
1720	GGGGAAGU	A	CUCAUGUG
1723	GAAGUACU	C	AUGUGUGA
1744	CGAGUGAU	U	GUGUCUAU
1749	GAUUGUGU	C	UAUGUGGA
1751	UUGUGUCU	A	UGUGGAUU
1759	AUGUGGAU	U	AUUUGCCC
1760	UGUGGAUU	A	UUUGCCCA
1762	UGGAUUUAU	U	UGCCCAUU
1763	GGAUUAUU	U	GCCCAUUA
1770	UUGCCCAU	U	AUUUAAUA
1771	UGCCCAUU	A	UUUAAUAA
1773	CCCAUUUAU	U	UAAUAAAG
1774	CCAUUUAUU	U	AAUAAAGA
1775	CAUUUAUUU	A	AUAAAGAG
1778	UAUUUAUU	A	AAGAGGAU
1787	AAGAGGAU	U	UGUCAAUU

Table AVII: Rabbit Stromelysin HH Ribozyme Sequence

nt. Position	Ribozyme Sequence
18	GCUGUCUU CUGAUGAGGCCGAAAGGCCGAA AUGCCUUG
29	CUCAGCUC CUGAUGAGGCCGAAAGGCCGAA AUGCUGUC
39	AUUGGCUU CUGAUGAGGCCGAAAGGCCGAA ACUCAGCU
61	GGUUGGAA CUGAUGAGGCCGAAAGGCCGAA AGUUUUA
63	AGGGUUGG CUGAUGAGGCCGAAAGGCCGAA AGAGUUUU
64	CAGGGUUG CUGAUGAGGCCGAAAGGCCGAA AAGAGUUU
75	CACAGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGGGU
93	GCUGAGCA CUGAUGAGGCCGAAAGGCCGAA AGCGCCAC
94	GGCUGAGC CUGAUGAGGCCGAAAGGCCGAA AAGCGCCA
98	GAUAGGCU CUGAUGAGGCCGAAAGGCCGAA AGCAAAGC
104	CCAGUGGA CUGAUGAGGCCGAAAGGCCGAA AGGCUGAG
106	AUCCAGUG CUGAUGAGGCCGAAAGGCCGAA AUAGGCTUG
122	CAUCCCUU CUGAUGAGGCCGAAAGGCCGAA AGGCUCCA
153	UGCUGAAG CUGAUGAGGCCGAAAGGCCGAA AGGUCCAU
154	UUGCUGAA CUGAUGAGGCCGAAAGGCCGAA AAGGUCCA
156	UAUUGCUG CUGAUGAGGCCGAAAGGCCGAA AGAAGGUC
157	AUAUUGCU CUGAUGAGGCCGAAAGGCCGAA AAGAAGGU
164	UUUCCAGA CUGAUGAGGCCGAAAGGCCGAA AUUGCUGA
166	GUUUUCCA CUGAUGAGGCCGAAAGGCCGAA AUAUUGCU
176	GGUUGUAG CUGAUGAGGCCGAAAGGCCGAA AGUUUUCC
179	CAAGGUUG CUGAUGAGGCCGAAAGGCCGAA AGUAGUUU
186	UCUUUUUC CUGAUGAGGCCGAAAGGCCGAA AGGUUGUA
206	UUUUAACA CUGAUGAGGCCGAAAGGCCGAA ACUGUUUC
207	CUUUUAC CUGAUGAGGCCGAAAGGCCGAA AACUGUUU
210	UUUCUUU CUGAUGAGGCCGAAAGGCCGAA ACAAACUG
211	CUUUCUU CUGAUGAGGCCGAAAGGCCGAA AACAAACU
226	AACAGGAC CUGAUGAGGCCGAAAGGCCGAA ACUGUCCU
229	AACAACAG CUGAUGAGGCCGAAAGGCCGAA ACUACUGU
234	UUUUUAA CUGAUGAGGCCGAAAGGCCGAA ACAGGACU
237	AUUUUUU CUGAUGAGGCCGAAAGGCCGAA ACAACAGG
238	GAUUUUU CUGAUGAGGCCGAAAGGCCGAA AACAACAG
246	AUUUCUUG CUGAUGAGGCCGAAAGGCCGAA AUUUUUU
263	AGCCAAGG CUGAUGAGGCCGAAAGGCCGAA ACUUCUGC
264	AAGCCAAG CUGAUGAGGCCGAAAGGCCGAA AACUUCUG
267	UCCAAGCC CUGAUGAGGCCGAAAGGCCGAA AGGAACUU
272	UCACCUCC CUGAUGAGGCCGAAAGGCCGAA AGCCAAGG
296	GGGUGUUG CUGAUGAGGCCGAAAGGCCGAA AGUCCAGC
315	GGCUUGCG CUGAUGAGGCCGAAAGGCCGAA AUCACCUC
336	ACAUCAGG CUGAUGAGGCCGAAAGGCCGAA ACGCCACA
337	AACAUCAG CUGAUGAGGCCGAAAGGCCGAA AACGCCAC
345	AAGUGACC CUGAUGAGGCCGAAAGGCCGAA ACAUCAGG

349	ACUGAAGU	CUGAUGAGGCCGAAAGGCCGAA	ACCAACAU
353	AGGUACUG	CUGAUGAGGCCGAAAGGCCGAA	AGUGACCA
354	AAGGUACU	CUGAUGAGGCCGAAAGGCCGAA	AAGUGACC
358	AGGGAAGG	CUGAUGAGGCCGAAAGGCCGAA	ACUGAAGU
362	UGCCAGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUACUG
363	GUGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUACU
391	AGUUAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUUG
396	CUGUAAGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUGAGU
400	AAUCCUGU	CUGAUGAGGCCGAAAGGCCGAA	AGUUAGGU
401	CAAUCCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGUUAGG
408	UAAUUCAC	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGUA
415	CGGUGUGU	CUGAUGAGGCCGAAAGGCCGAA	AUUCACAA
416	CCGGUGUG	CUGAUGAGGCCGAAAGGCCGAA	AAUUCACA
427	UCUUGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUCCGGUG
444	GCAGCAUC	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAUC
456	GCUUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAGC
466	GACCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUUUCU
474	UCCUCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACCUUCAG
490	CGUGAGUG	CUGAUGAGGCCGAAAGGCCGAA	AGUCACCU
495	GAGAACGU	CUGAUGAGGCCGAAAGGCCGAA	AGUGGAGU
500	UCCUGGAG	CUGAUGAGGCCGAAAGGCCGAA	ACGUGAGU
501	UUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	AACGUGAG
503	ACUCCUG	CUGAUGAGGCCGAAAGGCCGAA	AGAACGUG
512	CUCCUUCA	CUGAUGAGGCCGAAAGGCCGAA	ACUUCCTUG
531	GAGAUCAU	CUGAUGAGGCCGAAAGGCCGAA	AUGUCAGC
537	CCAAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUCAUUAU
539	CUCCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AGAUCAUU
541	GACUCCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAUCA
542	GGACUCCA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAUC
543	CGGACUCC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAGAU
549	UGUUCUCG	CUGAUGAGGCCGAAAGGCCGAA	ACUCCAAA
565	AGGAAUAA	CUGAUGAGGCCGAAAGGCCGAA	AUTCUCAU
566	AAGGAAUA	CUGAUGAGGCCGAAAGGCCGAA	AAUTCUCA
567	AAAGGAAU	CUGAUGAGGCCGAAAGGCCGAA	AAAUTCUC
568	AAAAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUTCUC
570	UCAAAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAAAAUC
571	AUCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAAU
574	UCCAUCAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAAUAA
575	GUCCAUC	CUGAUGAGGCCGAAAGGCCGAA	AAGGAAUA
576	GGUCCAUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAAU
594	UGAGCCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUC
595	AUGAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AACAUUUC
596	CAUGAGCC	CUGAUGAGGCCGAAAGGCCGAA	AAACAUUU
601	AUAAGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCCAAAA
607	AGGUGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAUGAG
608	CAGGUGCA	CUGAUGAGGCCGAAAGGCCGAA	AAGCAUGA
627	UCUCCAUI	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGG
628	AUTCUCAU	CUGAUGAGGCCGAAAGGCCGAA	AAUCCUG
644	CAUCAUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGGGCA

645	UCAUCAUC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGGGC
673	UCCUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUCCUUUG
688	AAGGAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGUUC
689	CAAGGAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGUU
690	ACAAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGGU
692	CAACAAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUUG
693	GCAACAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAUU
696	GCAGCAAC	CUGAUGAGGCCGAAAGGCCGAA	AGGAUATA
699	UGAGCAGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGGAA
706	AAGCUCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCAA
714	GAGUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGCUC AUG
722	GACCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGCCA
730	GUGAAACA	CUGAUGAGGCCGAAAGGCCGAA	ACCCAGGG
734	CCGAGUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGACCC
735	GCCGAGUG	CUGAUGAGGCCGAAAGGCCGAA	AACAGACC
736	GGCCGAGU	CUGAUGAGGCCGAAAGGCCGAA	AAACAGAC
740	GGUUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGUGAAAC
764	AGACUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAGC
771	GCGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGUA
773	AGGCGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGACUGGG
782	GGUCUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGCGUUG
783	AGGUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGGCGUU
800	AAAGGCGG	CUGAUGAGGCCGAAAGGCCGAA	ACCGGGCC
801	GAAAGGCG	CUGAUGAGGCCGAAAGGCCGAA	AACCGGGC
807	UCUUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCGGAA
808	AUCUUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCGGA
809	CAUCUUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGGCGG
811	AUCAUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAGGC
831	AGGGAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAUC
836	CAUAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGAUG
840	GGUCCAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGAUUG
842	CCGGUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGAU
860	UAUCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGGG
862	GUUAUCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGCAG
868	UCCAGAGU	CUGAUGAGGCCGAAAGGCCGAA	AUCAGGAG
872	GCACUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGUUAUCA
883	AGGUUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCACUC
894	CCUGGAGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGGUUC
898	AGAUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGGACAG
905	GGGUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGGA
918	UCACACAU	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGGU
928	CAGAUCUG	CUGAUGAGGCCGAAAGGCCGAA	AUCACACA
934	GAAGGACA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGAU
938	CAUCGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAUCU
941	UUGCAUCG	CUGAUGAGGCCGAAAGGCCGAA	AGGACAGA
942	AUUGCAUC	CUGAUGAGGCCGAAAGGCCGAA	AAGGACAG
951	AGAGUGCU	CUGAUGAGGCCGAAAGGCCGAA	AUUGCAUC
958	UCCCCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGCUGA
972	AAGAACAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUCC

973	AAAGAACA	CUGAUGAGGCCGAAAGGCCGAA	AAUUUCUC
977	CUUUAAAG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAAUU
978	UCUUUAAA	CUGAUGAGGCCGAAAGGCCGAA	AACAGAAU
980	UGUCUUUA	CUGAUGAGGCCGAAAGGCCGAA	AGAACAGA
981	CUGUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AAGAACAG
982	CCUGUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAACA
992	GCCAGAAA	CUGAUGAGGCCGAAAGGCCGAA	ACCUGUCU
994	GCGCCAGA	CUGAUGAGGCCGAAAGGCCGAA	AUACCUGU
995	UGC GCCAG	CUGAUGAGGCCGAAAGGCCGAA	AAUACCTUG
996	UUGCGCCA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACCTU
1007	UCCUGAGG	CUGAUGAGGCCGAAAGGCCGAA	ACTUUGCGC
1011	AGAAUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGGGACTUU
1017	GGUUCGAG	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGAG
1018	AGGUUCGA	CUGAUGAGGCCGAAAGGCCGAA	AAUCCUGA
1020	UCAGGUUC	CUGAUGAGGCCGAAAGGCCGAA	AGAAUCCU
1031	UCAAUAUG	CUGAUGAGGCCGAAAGGCCGAA	ACUCAGGU
1032	AUCAAUUG	CUGAUGAGGCCGAAAGGCCGAA	AACUCAGG
1033	GAUCAAU	CUGAUGAGGCCGAAAGGCCGAA	AAACUCAG
1036	AGAGAUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGAAACU
1037	AAGAGAU	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAAC
1041	AAUGAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUCAAUUG
1043	AGAAUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGAUCAAA
1045	CCAGAAUG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAUCA
1046	GCCAGAAU	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAU
1049	AUGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAGAG
1050	GAUGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAGA
1058	AAGGAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUGGCCAG
1060	UGAAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGCC
1062	GCUGAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAUUG
1063	UGCUGAAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAU
1066	CACUGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGAG
1067	CCACUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGGAAGA
1085	UAACUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCAGCA
1092	CUGCUAAU	CUGAUGAGGCCGAAAGGCCGAA	ACUUCAUA
1093	CCUGCUAA	CUGAUGAGGCCGAAAGGCCGAA	AACUUCAU
1095	UCCCUGCU	CUGAUGAGGCCGAAAGGCCGAA	AUAACUUC
1096	AUCCCUGC	CUGAUGAGGCCGAAAGGCCGAA	AAUAACUU
1105	GAAAACAG	CUGAUGAGGCCGAAAGGCCGAA	AUCCCUGC
1110	AAAAUGAA	CUGAUGAGGCCGAAAGGCCGAA	ACAGUAUC
1111	AAAAAUGA	CUGAUGAGGCCGAAAGGCCGAA	AACAGUAU
1112	UAAAAAUG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGUA
1113	UUAAAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAAACAGU
1116	CCUUUAAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAAAAC
1117	UCCUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAAA
1118	UUCUUUUA	CUGAUGAGGCCGAAAGGCCGAA	AAAUGAAA
1119	GUUCCUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAUGAA
1120	AGUUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAAUGA
1129	CCAGAACTU	CUGAUGAGGCCGAAAGGCCGAA	AGUUCUUU
1133	UGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	ACUGAGUU

1134	AUGGCCCA	CUGAUGAGGCCGAAAGGCCGAA	AACUGAGU
1143	UUUCCUCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGCCCA
1144	AUUUCCUC	CUGAUGAGGCCGAAAGGCCGAA	AAUGGCCC
1158	CCAGCUUG	CUGAUGAGGCCGAAAGGCCGAA	ACCUCAUU
1168	UCTUUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGCUU
1169	UUCUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AACCAGCU
1182	AGGGUGUG	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUCU
1195	UGAAGGGA	CUGAUGAGGCCGAAAGGCCGAA	ACCCAGGG
1196	UUGAAGGG	CUGAUGAGGCCGAAAGGCCGAA	AACCCAGG
1197	GUUGAAGG	CUGAUGAGGCCGAAAGGCCGAA	AAACCCAG
1201	UAUGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGGAAAC
1202	UUAUGGUU	CUGAUGAGGCCGAAAGGCCGAA	AAGGGAAA
1209	AUUUUUCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUUGA
1218	GCAGCAUC	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUCU
1230	UUUUCAGA	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAGC
1231	CUUAUCAG	CUGAUGAGGCCGAAAGGCCGAA	AAUGGCAG
1232	CCUUAUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAUGGCA
1237	CCUUUCCU	CUGAUGAGGCCGAAAGGCCGAA	AUCAGAAA
1256	CAAAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AUGUUUUC
1259	CCACAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGUAUGUU
1260	UCCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGUAUGU
1262	CUUCCACA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUUA
1263	UCUCCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGAAGUA
1277	ACCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUGUCU
1286	UCUCAUCA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCAG
1287	UUCUCAUC	CUGAUGAGGCCGAAAGGCCGAA	AACCUCCA
1304	GCUCCAGG	CUGAUGAGGCCGAAAGGCCGAA	ACUGUCUC
1319	GUCUGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGCCUGGC
1320	UGUCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCUGG
1321	AUGUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCUG
1330	UUCUGCUA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUGG
1332	UCTUCUGC	CUGAUGAGGCCGAAAGGCCGAA	AUAUGUCU
1343	UUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUUCU
1344	AUUCCTUG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUUC
1345	AAUUCCTU	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCUU
1353	UUUGGAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCCTUG
1354	CUUUGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAUUCCTU
1357	GAUCUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUAAUUC
1365	ACAGCAUC	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUGG
1374	GCTUCAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAUC
1375	UGCTUCA	CUGAUGAGGCCGAAAGGCCGAA	AACAGCAU
1376	AUGCTUCA	CUGAUGAGGCCGAAAGGCCGAA	AAACAGCA
1377	AAUGCTUC	CUGAUGAGGCCGAAAGGCCGAA	AAAACAGC
1385	AAAACCCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCTUCA
1386	AAAAACCC	CUGAUGAGGCCGAAAGGCCGAA	AAUGCTUC
1391	AAUAGAAA	CUGAUGAGGCCGAAAGGCCGAA	ACCCAAAU
1392	AAAUAGAA	CUGAUGAGGCCGAAAGGCCGAA	AACCCAAA
1393	GAAAUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAACCCAA
1394	AGAAAUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAACCCA

1395	AAGAAUA	CUGAUGAGGCCGAAAGGCCGAA	AAAAACCC
1397	UGAAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAAC
1399	ACUGAAG	CUGAUGAGGCCGAAAGGCCGAA	AUAGAAAA
1400	CACUGAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAAA
1401	CCACUGAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAA
1403	AUCCACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUAG
1404	GAUCCACU	CUGAUGAGGCCGAAAGGCCGAA	AAGAAUA
1412	ACUGUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUCCACUG
1414	CGACUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUCCAC
1415	CCGACUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUCCA
1421	CAAACUCC	CUGAUGAGGCCGAAAGGCCGAA	ACUGUGAA
1427	UUGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCGAC
1428	UUUGGGUC	CUGAUGAGGCCGAAAGGCCGAA	AACUCCGA
1458	CUCUUCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUGUGU
1459	GCUCUUC	CUGAUGAGGCCGAAAGGCCGAA	AACAUGUG
1460	UGCUCUUC	CUGAUGAGGCCGAAAGGCCGAA	AAACAUGU
1478	AACACUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGCUG
1479	UAACACUG	CUGAUGAGGCCGAAAGGCCGAA	AACCAGCU
1480	CUAACACU	CUGAUGAGGCCGAAAGGCCGAA	AAACCAGC
1486	CCCCUCCU	CUGAUGAGGCCGAAAGGCCGAA	ACACUGAA
1487	ACCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AACACUGA
1498	GCCUUCUA	CUGAUGAGGCCGAAAGGCCGAA	ACACCCCU
1500	GUGCCUUC	CUGAUGAGGCCGAAAGGCCGAA	AUACACCC
1519	UCAUUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCAU
1520	UUCAUUUA	CUGAUGAGGCCGAAAGGCCGAA	AACAUUCA
1521	GUUCAUUU	CUGAUGAGGCCGAAAGGCCGAA	AAACAUUC
1522	GGUUCAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAACAUU
1532	UGAACAAU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCAU
1535	UGUUGAAC	CUGAUGAGGCCGAAAGGCCGAA	AUUAGGUU
1538	AAGUGUUG	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUG
1539	UAAGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AACAAUUA
1546	AAAGUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUGA
1547	CAAAGUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUUG
1553	AACUCACA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUAA
1554	CAACUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCUA
1561	GCCACUUC	CUGAUGAGGCCGAAAGGCCGAA	ACUCACAA
1571	GAGAAAAU	CUGAUGAGGCCGAAAGGCCGAA	AGCCACTU
1574	CAGGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAGCCA
1575	GCAGGAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAGCC
1576	UGCAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AAAUGAGC
1577	AUGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAUGAG
1579	AUAUGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAUG
1586	UCACAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCAGGA
1602	AUGCUCGA	CUGAUGAGGCCGAAAGGCCGAA	AUUCCCAU
1604	UCAUGCUC	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCCC
1620	CAGUUAGA	CUGAUGAGGCCGAAAGGCCGAA	ACACAGUU
1622	UCCAGUUA	CUGAUGAGGCCGAAAGGCCGAA	AUACACAG
1624	AGUCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AGAUACAC
1633	GAUGUGCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAGU

1634	CGAUGUGC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCAG
1641	CCCGUAAC	CUGAUGAGGCCGAAAGGCCGAA	AUGUGCAA
1644	ACACCOGU	CUGAUGAGGCCGAAAGGCCGAA	ACGAUGUG
1645	AACACCCG	CUGAUGAGGCCGAAAGGCCGAA	AACGAUGU
1653	CCUGUUUG	CUGAUGAGGCCGAAAGGCCGAA	ACACCCGU
1654	GCCUGUUU	CUGAUGAGGCCGAAAGGCCGAA	AACACCCG
1670	UGCAAGCU	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCAG
1671	GUGCAAGC	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGCA
1675	UCAAGUGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUAAGC
1681	AUGUGAUC	CUGAUGAGGCCGAAAGGCCGAA	AGUGCAAG
1685	UUCCAUGU	CUGAUGAGGCCGAAAGGCCGAA	AUCAAGUG
1701	UCUCGUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCTU
1702	GUCUCGUG	CUGAUGAGGCCGAAAGGCCGAA	AAGCUCCT
1720	CACAUGAG	CUGAUGAGGCCGAAAGGCCGAA	ACTUCCCC
1723	UCACACAU	CUGAUGAGGCCGAAAGGCCGAA	AGUACUUC
1744	AUAGACAC	CUGAUGAGGCCGAAAGGCCGAA	AUCACUCG
1749	UCCACAU	CUGAUGAGGCCGAAAGGCCGAA	ACACAAUC
1751	AAUCCACA	CUGAUGAGGCCGAAAGGCCGAA	AGACACAA
1759	GGGCAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCACAU
1760	UGGGCAA	CUGAUGAGGCCGAAAGGCCGAA	AAUCCACA
1762	AAUGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUCCA
1763	UAAUGGGC	CUGAUGAGGCCGAAAGGCCGAA	AAUAAUCC
1770	UAUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCAA
1771	UUAUUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUGGGCA
1773	CUUUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAAUGGG
1774	UCUUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAUGG
1775	CUCUUUAU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAUG
1778	AUCCUCUU	CUGAUGAGGCCGAAAGGCCGAA	AUUAAAUA
1787	AAUUGACA	CUGAUGAGGCCGAAAGGCCGAA	AUCCUCUU

Table AVIII: Human Stromelysin Hairpin Ribozyme and Target Sequences

nt. Position	RZ	Substrate
66	CGCACAGC AGAA GUAGGA ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UCCUACU GUU GCUUGUGCG
82	GCUGAGCA AGAA GCCACG ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	CGUGGCA GUU UGCUUCAGC
91	AAUGGAUA AGAA GAGCAA ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UUGCUCA GCC UAUCCAUU
192	UCCUAACA AGAA GUUUC A ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UGAAACA GUU UGUUAGGA
220	UUUUUAAAC AGAA GGACCA ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UGGUCCU GUU GUUAAAAA
328	UGACCAAC AGAA GGAACU ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	AGUUCU GAU GUUGGUCA
412	UUUGGCAA AGAA GGUGUA ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UACACCA GAU UUGCCAAA
430	GCAGAAUC AGAA GCAUCU ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	AGAUGCU GUU GAUUCUGC
439	UUCUCAAC AGAA GAAUCA ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UGAUUCU GCU GUUGAGAA
442	GCUUUCUC AGAA GCAGAA ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UUUCUGU GUU GAGAAAGC
691	AUUUCAUG AGAA GCAACG ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	CGUUGCU GCU CAUGAAAU
775	CGAGUCAG AGAA GUGAGU ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	ACUCACA GAC CUGACUCG
780	GGAAACCG AGAA GGUCUG ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	CAGACCU GAC UCGGUUCC
786	ACAGGCGG AGAA GAGUCA ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UGACUCG GUU CCGCCTGU
791	UUGAGACA AGAA GAACCG ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	CGGUUCC GCC UGUUCUCAA
795	CAUCUUGA AGAA GGC GGA ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UCCGCCU GUC UCAAGAUG
822	CAUAGAGG AGAA GAAUGC ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	GCAUUA GUC CCUCUAUG
844	UCAGGGGA AGAA GGGGGA ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UCCCCCU GAC UCCCCUGA
880	UCUGGAGG AGAA GGUUCC ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	GGAACTU GUC CCUCCAGA
919	AAGGACAA AGAA GGAUCA ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UGAUCCU GCU UUGUCCUU
963	CUUUAAAG AGAA GGAUUU ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	AAAUCCU GAU CUUUAAAG
1360	UCUUCAAA AGAA GCAUCA ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UGAUGCU GUU UUUGAAGA
1407	CAAACTUC AGAA GUGAAG ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	CUUCACA GUU GGAGUUUG
1460	AUUUAGCC AGAA GUUACU ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	AGUAACA GCU GGCUUAAU
1570	ACAGCACA AGAA GGAGAA ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	UUUCUCU GCC UGUGCTUGU
1667	AGCUAAGC AGAA GOC CAU ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	AUGGGCU GCU GCUUAGCU
1670	GCAAGCUA AGAA GCAGCC ACCAGAGAAACACACACGUGUGGUACAUAUACCUUGUA	GGCUGCU GCU UAGCUUGC

Table AIX: Rabbit Hairpin Ribozyme and Target Sequences

nt. Position	Ribozyme Sequence	Substrate
77	CCAGGCAC AGAA GUAGCA ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	UGCUACU GCU GUGCGUGG
99	AGUGGAUA AGAA GAGCAA ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	UUGUCA GGC UAUCCACU
203	UUUUAACA AGAA GUUACA ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	UGAACA GUU UGUUAAA
231	UUUUUAC AGAA GGAUA ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	UAGUCCU GUU GUUAAAA
339	UGACCAAC AGAA GGAACG ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	CGUCCU GAU GUUGUCA
423	CUUGGCAG AGAA GGUGJG ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	GAACCG GAU CUGCCAG
441	GCAGCAUC AGAA GCAUCU ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	AGAUCCU GUU GAUGCUC
702	AGCUCAUG AGAA GCAACA ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	UGUUGU GCU CAUGAGCU
731	COGAGUGA AGAA GACCCA ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	UGGGUCU GUU UCAUCUGG
758	CUGGGUAC AGAA GCGCUU ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	AGGCGU GAU GUUACCCAG
768	GCGUUGUA AGAA GGGUAC ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	GUACCCA GUC UACAAAGC
786	CGGGCCAG AGAA GUGAAG ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	CUUCACA GAC CUGGCGCG
797	AAAGGGGG AGAA GGGCCA ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	UGGCGCG GUU CCGCGCUU
802	UUGAGAAA AGAA GAACCG ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	CGGUCC GGC UUUUCAA
849	GAGGCAGG AGAA GGUCCA ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	UGGACCG GGC CCUGGCUU
855	UCAGGAGA AGAA GGGGCC ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	GGCCCUU GGC UGUCCUGA
891	CCUGGAGG AGAA GGUUCC ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	GGAAUU GUC CCUCCAGG
930	AAGGACAG AGAA GGAUCA ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	UGAUCCA GAU CUGGCUUU
935	CAUCGAG AGAA GAUUG ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	CAGAUUU GUC CUUCGAG
974	CUUUAAAG AGAA GAUUUU ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	AAAUUUU GUU CUUUUWAG
1107	AAAUUGAA AGAA GUUCC ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	GGAUACU GUU UUCAUUUU
1130	UGGCCCCAG AGAA GAGUUC ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	GAACUCA GUU CUGGGCCA
1301	GUUCCAGG AGAA GUUUUU ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	AGAGACA GUC CCUGGAGC
1371	GUUUUAAA AGAA GCAUUG ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	CGAUUUU GUU UUUUWAGC
1418	CAAAUUC AGAA GUGAAG ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	CUUCACA GUC GGAGUUUG
1471	CUGAAAC AGAA GUUGUU ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	AGCAACA GCU GGUUUCAG
1663	AGCUAAGC AGAA GCUUGU ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	ACAGGCU GCU GCUUWAGCU
1666	GCAAGCUA AGAA GCAGCC ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	GGCUGCU GCU UAGCUUCC
1733	AAUACUUC AGAA GUACAA ACCAGAGAAACACACGJUGUGGJACAUUACCUUGGUA	UGUGACA GAC GAGUGAUU

Table BII: Human B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	AAACCCU C UGUAAAG	236	UGUGUGU U UUGUAAA
12	CCUCUGU A AAGUAAAC	237	GUGUGUU U UGUAAAC
17	GUAAAGU A ACAGAAG	238	UGUGUUU U GUAAACA
26	CAGAAGU U AGAAGGG	241	GUUUUGU A AACAUCA
27	AGAAGUU A GAAGGGG	247	UAAACAU C ACUGGAG
41	GAAUGU C GCCUCUC	258	GGAGGGU C UUCUACG
46	GUCGCCU C UCUGAAG	260	AGGGUCU U CUACGUG
48	CGCCUCU C UGAAGAU	261	GGGUCUU C UACGUGA
56	UGAAGAU U ACCCAAA	263	GUCUUCU A CGUGAGC
57	GAAGAUU A CCCAAAG	274	GAGCAAU U GGAUUGU
75	AAGUGAU U UGUCAUU	279	AUUGGAU U GUCAUCA
76	AGUGAUU U GUCAUUG	282	GAUUGU C AUCAGCC
79	GAUUUGU C AUUGCUU	285	UUGUCAU C AGCCCTUG
82	UUGUCAU U GCUUUAU	298	UGCCUGU U UUGCACC
86	CAUUGCU U UAUAGAC	299	GCCUGUU U UGCACCU
87	AUUGCUU U AUAGACU	300	CCUGUUU U GCACCUG
88	UUGCUUU A UAGACUG	322	CCCUGGU C UUACUUG
90	GCUUUAU A GACUGUA	324	CUGGUCU U ACUUGGG
97	AGACUGU A AGAAGAG	325	UGGUCUU A CUUGGGU
110	AGAACAU C UCAGAAG	328	UCUUACU U GGGUCCA
112	AACAUCU C AGAAGUG	333	CUUGGGU C CAAUUG
124	GUGGAGU C UUACCCU	339	UCCAAAU U GUUGGCU
126	GGAGUCU U ACCCUGA	342	AAAUUGU U GGCUUUC
127	GAGUCUU A CCCUGAA	347	GUUGGCU U UCACUUU
137	CUGAAAU C AAAGGAU	348	UUGGCUU U CACUUUU
145	AAAGGAU U UAAAGAA	349	UGGCUUU C ACUUUUG
146	AAGGAUU U AAAGAAA	353	UUUCACU U UUGACCC
147	AGGAUUU A AAGAAAA	354	UUCACUU U UGACCCU
163	GUGGAAU U UUUCUUC	355	UCACUUU U GACCCUA
164	UGGAAUU U UUCUUCA	362	UGACCCU A AGCAUCU
165	GGAAUUU U UCUUCAG	368	UAAGCAU C UGAAGCC
166	GAAUUUU U CUUCAGC	404	GGAACAU C ACCAUCC
167	AAUUUUU C UUCAGCA	410	UCACCAU C CAAGUGU
169	UUUUUCU U CAGCAAG	418	CAAGUGU C CAUACCU
170	UUUUCUU C AGCAAGC	422	UGUCCAU A CCUCAAU
187	UGAAACU A AAUCCAC	426	CAUACCU C AAUUUCU
191	ACUAAAU C CACAACC	430	CCUCAAU U UCUUUCA
200	ACAACCU U UGGAGAC	431	CUCAAUU U CUUUCAG
201	CAACCUU U GGAGACC	432	UCAAUUU C UUUCAGC
221	ACACCCU C CAAUCUC	434	AAUUUCU U UCAGCUC
226	CUCCAAU C UCUGUGU	435	AUUUCUU U CAGCUCU
228	CCAAUCU C UGUGUGU	436	UUUCUUU C AGCUCUU

441	UUCAGCU C UUGGUGC	782	GUGACGU U AUCAGUC
443	CAGCUCU U GGUGCUG	783	UGACGUU A UCAGUCA
457	GGCUGGU C UUUCUCA	785	ACGUUAU C AGUCAA
459	CUGGUCU U UCUCACU	789	UAUCAGU C AAAGCUG
460	UGGUCUU U CUCACUU	800	GCUGACU U CCCUACA
461	GGUCUUU C UCACUUC	801	CUGACUU C CCUACAC
463	UCUUUCU C ACUUCUG	805	CUUCCCU A CACCUAG
467	UCUCACU U CUGUUCA	811	UACACCU A GUUAUUC
468	CUCACUU C UGUUCAG	814	ACCUAGU A UAUCUGA
472	CUUCUGU U CAGGUGU	816	CUAGUAU A UCUGACU
473	UUCUGUU C AGGUGUU	818	AGUAUAU C UGACUUU
480	CAGGUGU U AUCCACG	824	UCUGACU U UGAAAUU
481	AGGUGUU A UCCACGU	825	CUGACUU U GAAAUUC
483	GUGUUAU C CACGUGA	831	UUGAAAU U CCAACUU
521	ACGCUGU C CUGUGGU	832	UGAAAUU C CAACUUC
529	CUGUGGU C ACAAUGU	838	UCCAACU U CUAUAU
537	ACAAUGU U UCUGUUG	839	CCAACUU C UAAUAU
538	CAAUGUU U CUGUUGA	841	AACUUCU A AUUAUAG
539	AAUGUUU C UGUUGAA	844	UUCUAU A UUAGAAG
543	UUUCUGU U GAAGAGC	846	CUAAUAU U AGAAGGA
562	ACAAACU C GCAUCUA	847	UAAUAU A GAAGGAU
567	CUCGCAU C UACUGGC	855	GAAGGAU A AUUUGCU
569	CGCAUCU A CUGGCAA	858	GGAUAAU U UGCUCAA
601	GCUGACU A UGAUGUC	859	GAUAAU U GCUCAAC
608	AUGAUGU C UGGGGAC	863	AUUUGCU C AACCUCU
622	CAUGAAU A UAUGGCC	869	UCAACCU C UGGAGGU
624	UGAAUAU A UGGCCCG	877	UGGAGGU U UCCAGA
635	CCCGAGU A CAAGAAC	878	GGAGGUU U UCCAGAG
651	GGACCAU C UUUGAUA	879	GAGGUUU U CCAGAGC
653	ACCAUCU U UGAUAUC	880	AGGUUUU C CAGAGCC
654	CCAUCUU U GAUAUCA	889	AGAGCCU C ACCUCUC
658	CUUUGAU A UCACUAA	894	CUACCUU C UCCUGGU
660	UUGAUUA C ACUAAUA	896	CACCUCU C CUGGUUG
664	UAUCACU A AUAACCU	902	UCCUGGU U GGAAAU
667	CACUAAU A ACCUCUC	920	GAAGAAU U AAAUGCC
672	AUAACCU C UCCAUG	921	AAGAAU A AAUGCCA
674	AACCUCU C CAUUGUG	930	AUGCCAU C AACACAA
678	UCUCCAU U GUGAUCC	942	CAACAGU U UCCCAAG
684	UUGUGAU C CUGGCUC	943	AACAGUU U CCAAGA
691	CCUGGCU C UGCGCCC	944	ACAGUUU C CCAAGAU
701	CGCCCAU C UGACGAG	952	CCAAGAU C CUGAAAC
716	GGCACAU A CGAGUGU	966	CUGAGCU C UAUGCUG
726	AGUGUGU U GUUCUGA	968	GAGCUCU A UGCUGUU
729	GUGUUGU U CUGAAGU	975	AUGCUGU U AGCAGCA
730	UGUUGUU C UGAAGUA	976	UGCUGUU A GCAGCAA
737	CUGAAGU A UGAAAAA	991	ACUGGAU U UCAAUAU
751	AGACGCU U UCAAGCG	992	CUGGAUU U CAAUAUG
752	GACGCUU U CAAGCGG	993	UGGAUUU C AAUAUGA
753	ACGCUUU C AAGCGGG	997	UUUCAAU A UGACAAC

1016	CACAGCU U CAUGUGU	1315	CAUGGAU C GUGGGGA
1017	ACAGCUU C AUGUGUC	1324	UGGGGAU C AUGAGGC
1024	CAUGUGU C UCAUCA	1334	GAGGCAU U CUUCCCU
1026	UGUGUCU C AUCAAGU	1335	AGGCAU U UUCCCUU
1029	GUCUCAU C AAGUAUG	1337	GCAUUCU U CCCUUA
1034	AUCAAGU A UGGACAU	1338	CAUUCUU C CCUUAAC
1042	UGGACAU U UAAGAGU	1342	CUUCCCU U AACAAAU
1043	GGACAUU U AAGAGUG	1343	UUCCCUU A ACAAUU
1044	GACAUUU A AGAGUGA	1350	AACAAAU U UAAGCUG
1054	AGUGAAU C AGACCUU	1351	ACAAAUU U AAGCUGU
1061	CAGACCU U CAACUGG	1352	CAAAUUU A AGCUGUU
1062	AGACCUU C AACUGGA	1359	AAGCUGU U UUAACCA
1072	CUGGAU A CAACCA	1360	AGCUGUU U UACCCAC
1090	AGAGCAU U UUCUGA	1361	GCUGUUU U ACCCACU
1091	GAGCAU U UCCUGAU	1362	CUGUUUU A CCCACUA
1092	AGCAUUU U CCUGAU	1369	ACCCACU A CCUCACC
1093	GCAUUUU C CUGAUAA	1373	ACUACCU C ACCUUCU
1099	UCCUGAU A ACCUGCU	1378	CUCACCU U CUUAAAA
1107	ACCUGCU C CCAUCCU	1379	UCACCUU C UUAAAAA
1112	CUCCCAU C CUGGGCC	1381	ACCUUCU U AAAAACC
1122	GGGCCAU U ACCUUA	1382	CCUUCUU A AAAACCU
1123	GGCCAUU A CCUUAU	1390	AAAACCU C UUUUAGA
1127	AUUACCU U AAUCUCA	1392	AACCUUCU U UCAGAUU
1128	UUACCUU A AUCUCAG	1393	ACCUUCU U CAGAUUA
1131	CCUUAU C UCAGUA	1394	CCUCUUU C AGAUUA
1133	UUAUUCU C AGUAAU	1399	UUCAGAU U AAGCUGA
1137	UCUCAGU A AAUGGAA	1400	UCAGAUU A AGCUGAA
1146	AUGGAAU U UUUGUGA	1412	GAACAGU U ACAAGAU
1147	UGGAAU U UUGUGAU	1413	AACAGUU A CAAGAUG
1148	GGAAUUU U UGUGAU	1429	CUGGCAU C CCUCUCC
1149	GAAUUUU U GUGAUU	1433	CAUCCCU C UCCUUUC
1155	UUGUGAU A UGCUGCC	1435	UCCUCUC C CUUUCUC
1169	CUGACCU A CUGCUUU	1438	CUCUCCU U UCUCCCC
1175	UACUGCU U UGCCCCA	1439	UCUCCUU U CUCCCCA
1176	ACUGCUU U GCCCCAA	1440	CUCCUUU C UCCCCAU
1214	GAGAGAU U GAGAAGG	1442	CCUUUCU C CCCAUU
1230	AAAGUGU A CGCCUG	1448	UCCCCAU A UGCAAU
1239	GCCUGU A UAACAGU	1455	AUGCAAU U UGCUUA
1241	CCUGUAU A ACAGUGU	1456	UGCAAU U GCUUAU
1249	ACAGUGU C CGCAGAA	1460	AUUUGCU U AAUGUA
1275	AAAAGAU C UGAAGGU	1461	UUUGCUU A AUGUAAC
1283	UGAAGGU A GCCUCCG	1466	UUAUGU A ACCUCUU
1288	GUAGCCU C CGUCAUC	1471	GUAACCU C UUCUUUU
1292	CCUCCGU C AUCUCUU	1473	AACCUUCU U CUUUUGC
1295	CCGUCAU C UCUUCUG	1474	ACCUCUU C UUUUGCC
1297	GUCAUCU C UUCUGGG	1476	CUCUUCU U UUGCCAU
1299	CAUCUCU U CUGGGAU	1477	UCUUCUU U UGCCAUG
1300	AUCUCUU C UGGGAUA	1478	CUUCUUU U GCCAUGU
1307	CUGGGAU A CAUGGAU	1486	GCCAUGU U UCCAUUC

1487	CCAUGUU U CCAUUCU
1488	CAUGUUU C CAUUCUG
1492	UUUCCAU U CUGCCAU
1493	UUCCAU C UGCCAUC
1500	CUGCCAU C UUGAAU
1502	GCCAUCU U GAAUUGU
1507	CUUGAAU U GUCUUGU
1510	GAAUUGU C UUGUCAG
1512	AUUGUCU U GUCAGCC
1515	GUCUUGU C AGCCAAU
1523	AGCCAAU U CAUUAUC
1524	GCCAAU C AUUAUCU
1527	AAUUCAU U AUCUAU
1528	AUUCAU A UCUAUA
1530	UCAUAU C UAUUAAA
1532	AUUAUCU A UUAACA
1534	UAUCUAU U AAACACU
1535	AUCUAU A AACACUA
1542	AAACACU A AUUUGAG

Table BIII: Human B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
8	CUUUACA CUGAUGAGGCCGAAAGGCCGAA AGGGUUU
12	GUUACUU CUGAUGAGGCCGAAAGGCCGAA ACAGAGG
17	CUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACUUUAC
26	CCCUUCU CUGAUGAGGCCGAAAGGCCGAA ACUUCUG
27	CCCUUC CUGAUGAGGCCGAAAGGCCGAA AACUUCU
41	GAGAGGC CUGAUGAGGCCGAAAGGCCGAA ACAUUUC
46	CUUCAGA CUGAUGAGGCCGAAAGGCCGAA AGGCGAC
48	AUCUUA CUGAUGAGGCCGAAAGGCCGAA AGAGGCG
56	UUUGGGU CUGAUGAGGCCGAAAGGCCGAA AUCUUA
57	CUUGGG CUGAUGAGGCCGAAAGGCCGAA AAUCUUC
75	AAUGACA CUGAUGAGGCCGAAAGGCCGAA AUCACUU
76	CAAUGAC CUGAUGAGGCCGAAAGGCCGAA AAUCACU
79	AAGCAAU CUGAUGAGGCCGAAAGGCCGAA ACAAUUC
82	AUAAAGC CUGAUGAGGCCGAAAGGCCGAA AUGACAA
86	GUCUAUA CUGAUGAGGCCGAAAGGCCGAA AGCAAUG
87	AGUCUAU CUGAUGAGGCCGAAAGGCCGAA AAGCAAU
88	CAGUCUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAA
90	UACAGUC CUGAUGAGGCCGAAAGGCCGAA AUAAAGC
97	CUCUUCU CUGAUGAGGCCGAAAGGCCGAA ACAGUCU
110	CUUCUGA CUGAUGAGGCCGAAAGGCCGAA AUGUUCU
112	CACUUCU CUGAUGAGGCCGAAAGGCCGAA AGAUGUU
124	AGGGUAA CUGAUGAGGCCGAAAGGCCGAA ACUCCAC
126	UCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGACUCC
127	UUCAGGG CUGAUGAGGCCGAAAGGCCGAA AAGACUC
137	AUCCUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCAG
145	UUCUUUA CUGAUGAGGCCGAAAGGCCGAA AUCCUUU
146	UUUCUUU CUGAUGAGGCCGAAAGGCCGAA AAUCCUU
147	UUUUCUU CUGAUGAGGCCGAAAGGCCGAA AAAUCCU
163	GAAGAAA CUGAUGAGGCCGAAAGGCCGAA AUUCCAC
164	UGAAGAA CUGAUGAGGCCGAAAGGCCGAA AAUUCCA
165	CUGAAGA CUGAUGAGGCCGAAAGGCCGAA AAAUUCC
166	GCUGAAG CUGAUGAGGCCGAAAGGCCGAA AAAAUUC
167	UGCUGAA CUGAUGAGGCCGAAAGGCCGAA AAAAAUU
169	CUUGCUG CUGAUGAGGCCGAAAGGCCGAA AGAAAAA
170	GCUUGCU CUGAUGAGGCCGAAAGGCCGAA AAGAAAA
187	GUGGAUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
191	GGUUGUG CUGAUGAGGCCGAAAGGCCGAA AUUUAGU
200	GUCUCCA CUGAUGAGGCCGAAAGGCCGAA AGGUUGU
201	GGUCUCC CUGAUGAGGCCGAAAGGCCGAA AAGGUUG
221	GAGAUUG CUGAUGAGGCCGAAAGGCCGAA AGGGUGU
226	ACACAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGAG

228	ACACACA	CUGAUGAGGCCGAAAGGCCGAA	AGAUUGG
236	UUUACAA	CUGAUGAGGCCGAAAGGCCGAA	ACACACA
237	GUUUACA	CUGAUGAGGCCGAAAGGCCGAA	AACACAC
238	UGUUUAC	CUGAUGAGGCCGAAAGGCCGAA	AAACACA
241	UGAUGUU	CUGAUGAGGCCGAAAGGCCGAA	ACAAAAC
247	CUCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AUGUUUA
258	CGUAGAA	CUGAUGAGGCCGAAAGGCCGAA	ACCCUCC
260	CACGUAG	CUGAUGAGGCCGAAAGGCCGAA	AGACCCU
261	UCACGUA	CUGAUGAGGCCGAAAGGCCGAA	AAGACCC
263	GCUCACG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAC
274	ACAAUCC	CUGAUGAGGCCGAAAGGCCGAA	AUUGCUC
279	UGAUGAC	CUGAUGAGGCCGAAAGGCCGAA	AUCCAUU
282	GGCUGAU	CUGAUGAGGCCGAAAGGCCGAA	ACAAUCC
285	CAGGGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGACAA
298	GGUGCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCA
299	AGGUGCA	CUGAUGAGGCCGAAAGGCCGAA	AACAGGC
300	CAGGUGC	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
322	CAAGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
324	CCCAAGU	CUGAUGAGGCCGAAAGGCCGAA	AGACCAG
325	ACCCAAG	CUGAUGAGGCCGAAAGGCCGAA	AAGACCA
328	UGGACCC	CUGAUGAGGCCGAAAGGCCGAA	AGUAAGA
333	CAAUUUG	CUGAUGAGGCCGAAAGGCCGAA	ACCCAAG
339	AGCCAAC	CUGAUGAGGCCGAAAGGCCGAA	AUUUGGA
342	GAAAGCC	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUU
347	AAAGUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCCAAC
348	AAAAGUG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAA
349	CAAAAGU	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
353	GGGUCAA	CUGAUGAGGCCGAAAGGCCGAA	AGUGAAA
354	AGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AAGUGAA
355	UAGGGUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGUGA
362	AGAUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGGUCA
368	GGCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUA
404	GGAUGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGUCC
410	ACACUUG	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGA
418	AGGU AUG	CUGAUGAGGCCGAAAGGCCGAA	ACACUUG
422	AUUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGGACA
426	AGAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGU AUG
430	UGAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGAGG
431	CUGAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGAG
432	GCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGA
434	GAGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUU
435	AGAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGAAAU
436	AAGAGCU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAA
441	GCACCAA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAA
443	CAGCACC	CUGAUGAGGCCGAAAGGCCGAA	AGAGCUG
457	UGAGAAA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGCC
459	AGUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGACCAG
460	AAGUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGACCA
461	GAAGUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGACC

463 CAGAAGU CUGAUGAGGCCGAAAGGCCGAA AGAAAGA
467 UGAACAG CUGAUGAGGCCGAAAGGCCGAA AGUGAGA
468 CUGAACA CUGAUGAGGCCGAAAGGCCGAA AAGUGAG
472 ACACCU CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
473 AACACCU CUGAUGAGGCCGAAAGGCCGAA AACAGAA
480 CGUGGAU CUGAUGAGGCCGAAAGGCCGAA ACACCU CUG
481 ACGUGGA CUGAUGAGGCCGAAAGGCCGAA AACACCU
483 UCACGUG CUGAUGAGGCCGAAAGGCCGAA AUAACAC
521 ACCACAG CUGAUGAGGCCGAAAGGCCGAA ACAGCGU
529 ACAUUGU CUGAUGAGGCCGAAAGGCCGAA ACCACAG
537 CAACAGA CUGAUGAGGCCGAAAGGCCGAA ACAUUGU
538 UCAACAG CUGAUGAGGCCGAAAGGCCGAA AACAUUG
539 UUCAACA CUGAUGAGGCCGAAAGGCCGAA AAACAUU
543 GCUCUUC CUGAUGAGGCCGAAAGGCCGAA ACAGAAA
562 UAGAUGC CUGAUGAGGCCGAAAGGCCGAA AGUUUGU
567 GCCAGUA CUGAUGAGGCCGAAAGGCCGAA AUGCGAG
569 UUGCCAG CUGAUGAGGCCGAAAGGCCGAA AGAUGCG
601 GACAUCA CUGAUGAGGCCGAAAGGCCGAA AGUCAGC
608 GUCCCCA CUGAUGAGGCCGAAAGGCCGAA ACAUCAU
622 GGCCAUU CUGAUGAGGCCGAAAGGCCGAA AUUCAUG
624 CGGGCCA CUGAUGAGGCCGAAAGGCCGAA AUAUUCA
635 GUUCUUG CUGAUGAGGCCGAAAGGCCGAA ACUCGGG
651 UAUCAAA CUGAUGAGGCCGAAAGGCCGAA AUGGUCC
653 GAUAUCA CUGAUGAGGCCGAAAGGCCGAA AGAUGGU
654 UGAUAUC CUGAUGAGGCCGAAAGGCCGAA AAGAUGG
658 UUAGUGA CUGAUGAGGCCGAAAGGCCGAA AUCAAAG
660 UAUUAGU CUGAUGAGGCCGAAAGGCCGAA AUAUCA
664 AGGUUAU CUGAUGAGGCCGAAAGGCCGAA AGUGAUA
667 GAGAGGU CUGAUGAGGCCGAAAGGCCGAA AUUAGUG
672 CAAUGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUAU
674 CACAAUG CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
678 GGAUCAC CUGAUGAGGCCGAAAGGCCGAA AUGGAGA
684 GAGCCAG CUGAUGAGGCCGAAAGGCCGAA AUCACAA
691 GGGCGCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
701 CUCGUCA CUGAUGAGGCCGAAAGGCCGAA AUGGGCG
716 ACACUCG CUGAUGAGGCCGAAAGGCCGAA AUGUGCC
726 UCAGAAC CUGAUGAGGCCGAAAGGCCGAA ACACACU
729 ACUUCAG CUGAUGAGGCCGAAAGGCCGAA ACAACAC
730 UACUUCA CUGAUGAGGCCGAAAGGCCGAA AACACA
737 UUUUCA CUGAUGAGGCCGAAAGGCCGAA ACUUCAG
751 CGCUUGA CUGAUGAGGCCGAAAGGCCGAA AGCGUCU
752 CCGCUUG CUGAUGAGGCCGAAAGGCCGAA AAGCGUC
753 CCCGCUU CUGAUGAGGCCGAAAGGCCGAA AAAGCGU
782 GACUGAU CUGAUGAGGCCGAAAGGCCGAA ACGUCAC
783 UGACUGA CUGAUGAGGCCGAAAGGCCGAA AACGUCA
785 UUUGACU CUGAUGAGGCCGAAAGGCCGAA AUAACGU
789 CAGCUUU CUGAUGAGGCCGAAAGGCCGAA ACUGAUA
800 UGUAGGG CUGAUGAGGCCGAAAGGCCGAA AGUCAGC
801 GUGUAGG CUGAUGAGGCCGAAAGGCCGAA AAGUCAG

805	CUAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGGAAG
811	GAUAUAC	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUA
814	UCAGAUU	CUGAUGAGGCCGAAAGGCCGAA	ACUAGGU
816	AGUCAGA	CUGAUGAGGCCGAAAGGCCGAA	AUACUAG
818	AAAGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUACU
824	AAUUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCAGA
825	GAAUUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCAG
831	AAGUUGG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCA
832	GAAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUUUCA
838	AUAUUAG	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGA
839	AAUAUUA	CUGAUGAGGCCGAAAGGCCGAA	AAGUUGG
841	CUAAUUA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUU
844	CUUCUAA	CUGAUGAGGCCGAAAGGCCGAA	AUUAGAA
846	UCCUUCU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUG
847	AUCCUUC	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUA
855	AGCAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCUUC
858	UUGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUUAUCC
859	GUUGAGC	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUC
863	AGAGGUU	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAU
869	ACCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
877	UCUGGAA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
878	CUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	AACCUCC
879	GCUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAACCUC
880	GGCUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAAACCU
889	GAGAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCUCU
894	ACCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUGAG
896	CAACCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUG
902	AUUUUCC	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGA
920	GGCAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCUUC
921	UGGCAUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUU
930	UUGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAU
942	CUUGGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUGUUG
943	UCUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AACUGUU
944	AUCUUGG	CUGAUGAGGCCGAAAGGCCGAA	AAACUGU
952	GUUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGG
966	CAGCAUA	CUGAUGAGGCCGAAAGGCCGAA	AGCUCAG
968	AACAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAGCUC
975	UGCUGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAU
976	UUGCUGC	CUGAUGAGGCCGAAAGGCCGAA	AACAGCA
991	AUAUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCAGU
992	CAUAUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUCCAG
993	UCAUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAUCCA
997	GUUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUGAAA
1016	ACACAUG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGUG
1017	GACACAU	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGU
1024	UUGAUGA	CUGAUGAGGCCGAAAGGCCGAA	ACACAUG
1026	ACUUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGACACA
1029	CAUACUU	CUGAUGAGGCCGAAAGGCCGAA	AUGAGAC
1034	AUGUCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUUGAU

1042	ACUCUUA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCCA
1043	CACUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUGUCC
1044	UCACUCU	CUGAUGAGGCCGAAAGGCCGAA	AAAUGUC
1054	AAGGUCU	CUGAUGAGGCCGAAAGGCCGAA	AUUCACU
1061	CCAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUG
1062	UCCAGUU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCU
1072	UUGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAG
1090	UCAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGCUCU
1091	AUCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGCUC
1092	UAUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAAUGCU
1093	UUAUCAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAUGC
1099	AGCAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUCAGGA
1107	AGGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGU
1112	GGCCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGGGAG
1122	UUAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGCCC
1123	AUUAAGG	CUGAUGAGGCCGAAAGGCCGAA	AAUGGCC
1127	UGAGAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGUAAU
1128	CUGAGAU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAA
1131	UUACUGA	CUGAUGAGGCCGAAAGGCCGAA	AUUAAGG
1133	AUUUACU	CUGAUGAGGCCGAAAGGCCGAA	AGAUUAA
1137	UUCCAUU	CUGAUGAGGCCGAAAGGCCGAA	ACUGAGA
1146	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAU
1147	AUCACAA	CUGAUGAGGCCGAAAGGCCGAA	AAUUCCA
1148	UAUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUCC
1149	AUAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUUC
1155	GGCAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUCACAA
1169	AAAGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
1175	UGGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGUA
1176	UUGGGGC	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGU
1214	CCUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AUCUCUC
1230	CAGGGCG	CUGAUGAGGCCGAAAGGCCGAA	ACACUUU
1239	ACUGUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGGC
1241	ACACUGU	CUGAUGAGGCCGAAAGGCCGAA	AUACAGG
1249	UUCUGCG	CUGAUGAGGCCGAAAGGCCGAA	ACACUGU
1275	ACCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUU
1283	CGGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACCUUCA
1288	GAUGACG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUAC
1292	AAGAGAU	CUGAUGAGGCCGAAAGGCCGAA	ACGGAGG
1295	CAGAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUGACGG
1297	CCCAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAC
1299	AUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAUG
1300	UAUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAU
1307	AUCCAUG	CUGAUGAGGCCGAAAGGCCGAA	AUCCAG
1315	UCCCCAC	CUGAUGAGGCCGAAAGGCCGAA	AUCCAUG
1324	GCCUCAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCCCA
1334	AGGGAAG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCUC
1335	AAGGGAA	CUGAUGAGGCCGAAAGGCCGAA	AAUGCCU
1337	UUAAGGG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUGC
1338	GUUAAGG	CUGAUGAGGCCGAAAGGCCGAA	AAGAAUG

1342	AUUUGUU	CUGAUGAGGCCGAAAGGCCGAA	AGGGAAG
1343	AAUUUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGGGAA
1350	CAGCUUA	CUGAUGAGGCCGAAAGGCCGAA	AUUUGUU
1351	ACAGCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUUGU
1352	AACAGCU	CUGAUGAGGCCGAAAGGCCGAA	AAAUUUG
1359	UGGGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
1360	GUGGGUA	CUGAUGAGGCCGAAAGGCCGAA	AACAGCU
1361	AGUGGGU	CUGAUGAGGCCGAAAGGCCGAA	AAACAGC
1362	UAGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAACAG
1369	GGUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGGU
1373	AGAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGU
1378	UUUUAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGAG
1379	UUUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAGGUGA
1381	GGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAGGU
1382	AGGUUUU	CUGAUGAGGCCGAAAGGCCGAA	AAGAAGG
1390	UCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUUU
1392	AAUCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUU
1393	UAAUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGAGGU
1394	UUAUCU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAGG
1399	UCAGCUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAA
1400	UUCAGCU	CUGAUGAGGCCGAAAGGCCGAA	AAUCUGA
1412	AUCUUGU	CUGAUGAGGCCGAAAGGCCGAA	ACUGUUC
1413	CAUCUUG	CUGAUGAGGCCGAAAGGCCGAA	AACUGUU
1429	GGAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
1433	GAAAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGGAUG
1435	GAGAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGA
1438	GGGGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGAGAG
1439	UGGGGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGAGA
1440	AUGGGGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAG
1442	AUAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGAAAGG
1448	AAUUGCA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGGA
1455	UUAAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUUGCAU
1456	AUUAAGC	CUGAUGAGGCCGAAAGGCCGAA	AAUUGCA
1460	UUACAUU	CUGAUGAGGCCGAAAGGCCGAA	AGCAAUU
1461	GUUACAU	CUGAUGAGGCCGAAAGGCCGAA	AAGCAAA
1466	AAGAGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUAA
1471	AAAAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUAC
1473	GCAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUU
1474	GGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGGU
1476	AUGGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAG
1477	CAUGGCA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAGA
1478	ACAUGGC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
1486	GAAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAUGGC
1487	AGAAUGG	CUGAUGAGGCCGAAAGGCCGAA	AACAUGG
1488	CAGAAUG	CUGAUGAGGCCGAAAGGCCGAA	AAACAUG
1492	AUGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGGAAA
1493	GAUGGCA	CUGAUGAGGCCGAAAGGCCGAA	AAUGGAA
1500	AAUUCAA	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAG
1502	ACAAUUC	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGC

1507	ACAAGAC	CUGAUGAGGCCGAAAGGCCGAA	AUUCAAG
1510	CUGACAA	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUC
1512	GGCUGAC	CUGAUGAGGCCGAAAGGCCGAA	AGACAAU
1515	AUUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAAGAC
1523	GAUAAUG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCU
1524	AGAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGC
1527	AAUAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUGAAU
1528	UAAUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAU
1530	UUUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUGA
1532	UGUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AGAUAAU
1534	AGUGUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAGAUA
1535	UAGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAU
1542	CUCAAAU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUU

Table BIV: Mouse B7-1 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	GaGUuUU a UACcUcA	108	CaUcUUU a GCAuCUG
10	guUuuAU A CCUCAAU	108	CAUcUUU a gcaUCUG
10	GUuUUaU a ccuCAAU	131	aUGCCAU C caGgcUU
14	uAUacCU c aAUAGAC	142	gCUuCUU U uUCuaCA
18	CcucAAU A gaCUCUu	142	gCUUCUU u UUcUaCa
18	CCUCaaU a gaCUCUU	143	CUuCUUU u UCuaCAU
18	CcUcAAU a GaCUcuU	143	CuUcUuU u uCuAcAU
23	AuaGaCU c uUACuaG	143	CUUCUUU U uCuAcaU
25	AGACuCU U aCuAGuu	143	cUUCuUU u UCUAcau
26	GACuCUU a CuAGuuu	144	UuCuUuU U cUaCAuC
29	UCUUACU a GuuUCuc	144	UuCuuuU u cUaCAUC
29	UcUuACU a gUuuCuC	144	UUCuuUU u cuaCAUC
29	UCUUaCU a guUUCUc	147	uUUUuCU a cAuCUCU
29	UCuuaCU a gUUUCUC	153	uAcAuCU C ugUUUCU
34	CUaGUuU c UCUuuuU	165	uCUCgAU U UuUgUGa
34	CUAGUuU c UCUuuuU	165	uCUCgAU u UuuGUGa
34	cUAgUuU c uCuUuUU	165	ucucgAU U UUUGUGA
40	ucuCUuU U UCAGgUU	166	CUCgAUU U uUgUgAG
41	cUCUuUU u caGGuUg	167	uCgAUuU u UGUGaGc
41	cuCUuUU U CAGgUUG	167	ucGauUU U UGUgAgC
42	uCUuUUU C AGgUUGu	167	UCgAUUU u UgUgAGC
56	UGAAACU c AAACuuC	168	cGAUuuU u gUgAGCC
56	UGAAAcU C aAcCUUC	168	cgAUUUU U GUGAgcc
62	uCAACCU U caaAGAC	197	GCUccAU u GgCUcUA
62	UCaAcCU U CaAAgAc	202	aUUGGCU c UagaUUc
62	UCAACCU u caaAGac	208	UCuAgAU U ccUGGCU
63	CAACCUU c aaAGACa	216	CCUGGCU u UcCcCau
73	aGAcAcU c UGuUCCa	217	cUGGCUU U CcCcaUc
77	acUCUgU u cCAuUUC	217	cUgGCuU u CccCAUC
78	CucUGUU c CauUUCU	217	CUGGCuU u CCcCauC
83	UucCAuU U CUGUggA	218	UGGcuUU c ccCaUCA
93	GUggAcU A AuAGgAu	218	UGGCUUU C cCcaUca
93	gUgGacU a AUAGgaU	218	UGgCuUU c cCcaUCA
93	gUGgAcU a AuAGGAU	218	ugGcUUU c CCCAUcA
96	GACuAAU a GGAUcaU	224	UCcCCAU c aUGuUCu
96	gacuAAU a gGAuCaU	224	UccCCAU c aUGuucU
101	AUaGGAU c aUCuUuA	230	UCAugUU C UccAAAag
104	GGAuCAU C uuuAgCa	232	AuGUUcU C CAaAGCa
104	GGAuCAU C UUUagcA	232	AUGuUcU c caaAGCA
106	AuCAUCU U UagcAUC	232	AugUUCU c cAAAagCa
107	UcAuCuU u AGCAUCU	241	AAAGcAU c UgAAGcu
107	uCaUCUU u AgcAuCU	241	aAAGCAU C UGAAGCu

241	AAAgcAU C	UGAAGcU	556	ACCuACU c	uCUuAuC
249	UGAAGcU A	UGGCuuG	556	AcCuAcU c	ucUUAUC
264	CAAuUgU c	AGuUGaU	560	AcUcUCU U	aUCAuCC
287	CACaCU c	CUcaagU	561	cUCuCUU a	UcAuCCU
295	CUCaAgU u	UCcaUGU	561	cuCUcuU a	uCAUCCU
295	cuCAaGU U	UCCAUGu	561	CUCUCuU a	UCauCCu
296	uCAAgUU u	ccAUgUc	566	UUaUcAU C	CUGGgcC
297	CAAGUuU C	CAUguCc	566	uUauCAU C	CUGGGCC
297	CAAGuuU c	cAUGuCC	581	UGGuCcU U	UcAGAcc
314	GGCUcaU u	cUUCUCu	583	gucCUUU C	AgaCcGG
314	GgcUcAU U	CUUCuCU	583	GuCcUUU c	AGAccGg
315	GcuCAUU c	UuCUcuU	598	GGCACAU A	CagcUGU
315	gcuCAUU C	UUCuCUU	608	gcUGUGU c	GUUCaaA
317	uCAUUCU U	CuCUUug	611	GUGUcgU u	CAaaaGA
318	CAUUCUU C	uCUUugu	611	GUGUcGU U	CaaAAGa
318	CAUuCuU C	UCuUUgu	612	UGUcGUU C	aaAAGaA
320	uUCUUCU c	uuUGuGC	641	aUGaAGU u	aaACaCU
320	UUCuuCU C	UUuGUGC	649	AAAcacU U	GGCUUUa
322	CuuCUCU U	uGUGCUG	649	AaaCAcU U	gGCUUuA
322	CUucuC U	UgUGCUG	655	UUggcuU u	AGUAAAg
323	UUcuCUU u	gUGcugC	656	UGgcUUU a	GUAAAgU
336	gcUGAUU c	GUcuUUC	659	CuUuaGU A	AAGUugu
341	uUCGuCU u	UCacAAG	664	GUaAaGU U	gUCcaUC
341	UUCgucU u	UcAcAAG	667	AaGUUGU C	caUCAAA
342	UcGUCUU U	CaCAagU	671	UgUCcaU C	AAAGCUG
343	cgucUuU C	AcAAGUG	682	gCUGAcU u	CuCuACC
343	cGuCuUU c	AcaAGUG	682	GCUGACU U	CuCUACc
352	caAGUGU C	uuCAGAu	682	GCUGacU U	cuCuACc
355	gUgUcUU C	AGaUGUU	683	CUGACUU C	uCUACcC
382	UCcaAGU c	AgUGaAA	683	CUGACUU c	ucuAccC
408	gCUGCcU U	GCCguuA	685	gACUuCU c	UaCCCCc
414	UUGccgU U	aCAACUc	685	gaCUucU c	UACCCcC
414	UUGCCgU u	ACAACUc	687	CUUCuCU A	CcCCcAa
421	UaCAAcU c	uCcUcAU	698	ccAACAU a	ACUGagu
426	CUCuCCU c	aUgAAgA	698	CCaCAU A	ACuGaGU
439	GaUGAgU c	UGAaGaC	718	AAcCCaU C	UGcAgAc
452	acCGaAU C	UACUGGC	718	aaCCCAU c	UGCagac
454	CGaAUCU A	CUGGCAA	729	AGACacU A	AaAgGAU
484	GuGCUGU c	UGucaUU	729	agAcAcU A	aaAGGAU
484	GugCUGU c	UguCAuU	729	agACAcU a	AaAgGAU
488	ugUcUGU C	AUUGCUg	737	aAAGGAU u	AccUGCU
503	gGAAacU A	aaAGuGu	737	aaAGgAU U	AccUGCu
503	ggAAAcU a	AAagUGU	737	aaagGAU u	ACCUGCU
520	CCCGAGU A	uAAGAAC	745	aCCUGcU U	UGCuuCc
535	cGGAcUU U	aUaUGAc	745	accUGcU u	UGCUuCC
536	GGAcUUU a	UaUGAcA	759	cGggGgU U	uCCCAAA
538	AcUuUUAU a	UGACaac	759	cGgGGGU u	UcCcAaa
553	acuACCU a	cUCUcUU	759	cGGgGGU U	UcCCAaA
553	AcUaCcU a	cUCUcUU	760	GggGgUU u	CCCAAG

760	gGGgGUU u cCCAAag	1060	aaaUgcU u cUGUaAG
760	GGgGGUU U cCCAAaAG	1060	AAAugCU u cUgUaAG
761	GgGGUUU c CCAaAGC	1061	AAUGcUU C UGUaagc
771	aaAgccU C GCuUCUC	1080	AagcugU u UCAGAAG
771	AaAGCCU C gCuUCUC	1080	AAGCUGU U UcAgaag
776	CUCgCUU C UcUUGgu	1081	AgCuGUU u CAgAga
776	CUCgCUU C UCuUGGU	1121	acAGcCU U ACCuUcg
778	CgCuUCU C uUGGUUG	1121	AcAgCCU u aCCuUcG
784	UCuUGGU U GGAAAAU	1121	ACagCCU u ACCUUCg
803	GAGaaUU A CCugGcA	1122	CaGcCUU a cCUUCgG
803	gAGAAUU A ccUGgCA	1126	CUuACCU u CgGgccU
803	gagAaUU a CCUGgcA	1127	UUaCcUU c ggGcCUG
812	cUGgCAU C AAuACgA	1127	UuACcUU c GggCCUG
812	CUGGcAU c aAuaCgA	1144	GaagCAU U AgCUgAA
816	caUCAAU A cGACAAu	1144	gaAGcaU u AGCUGAA
816	cAUCaAU a cgACAAu	1145	aAgcAUU a GCUgAAC
824	CgACAAU U UCCCCagG	1160	AGAcCgU c UUCCUuu
825	gACAAUU U CCCAgGA	1162	AcCgUCU u CcUUuaG
826	ACAAUUU C CCagGAU	1163	ccGUCUU c CUUuaGU
834	CCagGAU C CUGAAuC	1167	cUUCcUU u AGuUCUU
841	CcUGaaU C ugAAUUG	1177	uUCUUCU c UguCCAU
841	cCUGaaU c UGAAuUg	1181	UCuCugU C CAuGUGg
850	gAAuUGU A CaCCaUu	1181	ucUCUGU c CAuGUGg
869	gccAaCU a gAUuUCA	1192	gUGGGAU A CAUGGua
869	GCCAaCU a GAuUUca	1199	aCaUGGU a UUAUGUG
869	GCCAaCU a gaUuUCA	1201	AuGgUaU u aUGUGGc
873	acUaGAU u UCAaUAc	1210	ugUGGcU C aUGAGGu
873	ACUaGAU U UCAAUAc	1210	UGuGGcU C AUGAGGu
874	CUaGAUU U CAAUAcG	1223	GUacAAU c UUUCUUu
875	UaGAUUU C AAUAcGA	1225	ACAAUcU U UCuUuca
885	UAcgACU c gcAACCc	1225	ACAAuCU u uCuUuca
899	ACACCau u aAgUgUC	1226	caAuCUU u cUuUCAG
899	ACACCaU u AaGUGUC	1227	aAucUUU c uUUCAGC
906	UaaGUGU c UcaUuAA	1227	AAucuuU C UUUCAGc
906	uAaGUGU C UCAUuAA	1227	AAuCUuU c uUucaGC
908	aGUGUCU C AUuAAaU	1229	ucUUUCU U UCAGCaC
911	GUCUCAU u AAaUAUG	1230	cUUUCUU U CAGCaCc
916	AUuAaaU a UGGaGAU	1252	cUgAUCU u UcggACA
916	AUuAAaU A UGGagAU	1274	acaAGAU a gAGuUaA
943	gAGgaCU U CACcUGG	1310	UGAgGaU u uCuUuCc
944	AGgaCUU C AcCUGGg	1312	aGgAUUU c UuUcCAu
1001	UGCUCUU u GggGCag	1314	gAUUUcU u UcCAuCA
1034	CAGucGU c gUCauCG	1316	UUUcUuU c CAuCAgG
1037	UcGUCgU C AuCguUG	1320	UUUcCaU C AGgAAGC
1043	uCAUCgU U GuCAUCA	1320	UUUCcaU c aggaAGC
1046	ucgUUGU c AuCAUCA	1339	GgCAagU u UgCUGGG
1049	uUguCaU c AuCAAAU	1355	cUuUgAU U GCUUgAU
1060	aaaUGcU U CUGUaag	1437	gUGguaU A aGAAAAA
1060	AAaUgcU u cUgUaAG	1437	gUggUAU a AGAAAAA

1475	gCCUAGU	c	UuaCUGc
1477	CUaGUCU	U	ACUgcaa
1487	ugCAaCU	U	gAUaUGU
1491	AcuUGAU	a	UGUCAUg
1491	aCUUgaU	a	UGuCAUG
1505	gUUUGgU	U	ggUGUcu
1530	uGCCcUU	u	uCUgAAg
1531	GCccUUU	u	CUGAagA
1532	CcCuUuU	C	UGAAGAg
1532	CcCuuuU	C	UGAaGAG
1644	CUaUGGU	u	gggAUGU
1652	ggGAuGU	a	AaAAcGG
1652	GgGAugU	a	aAaAcGG
1670	aUaAUaU	a	AaUAuUA
1674	uAUAAAU	a	UuAaaUa
1676	UaAaUAU	u	aAaUAAA
1677	AAaUAUU	a	AAuaAAA
1677	AaaUAUU	A	AAuAaaa
1694	AGagUaU	u	gAGcAAA

Table BV: Mouse B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences		
8	UGAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AAAACUC
10	AUUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAAAAC
10	AUUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAAAAC
14	GUCUAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUAU
18	AAGAGUC	CUGAUGAGGCCGAAAGGCCGAA	AUUGAGG
18	AAGAGUC	CUGAUGAGGCCGAAAGGCCGAA	AUUGAGG
18	AAGAGUC	CUGAUGAGGCCGAAAGGCCGAA	AUUGAGG
23	CUAGUAA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUAU
25	AACUAGU	CUGAUGAGGCCGAAAGGCCGAA	AGAGUCU
26	AAACUAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAGUC
29	GAGAAAC	CUGAUGAGGCCGAAAGGCCGAA	AGUAAGA
29	GAGAAAC	CUGAUGAGGCCGAAAGGCCGAA	AGUAAGA
29	GAGAAAC	CUGAUGAGGCCGAAAGGCCGAA	AGUAAGA
29	GAGAAAC	CUGAUGAGGCCGAAAGGCCGAA	AGUAAGA
34	AAAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAACUAG
34	AAAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAACUAG
34	AAAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAACUAG
40	AACCUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAGA
41	CAACCUG	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAG
41	CAACCUG	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAG
42	ACAACCU	CUGAUGAGGCCGAAAGGCCGAA	AAAAAGA
56	GAAGGUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCA
56	GAAGGUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCA
62	GUCUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
62	GUCUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
62	GUCUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
63	UGUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUUG
73	UGGAACA	CUGAUGAGGCCGAAAGGCCGAA	AGUGUCU
77	GAAAUGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAGU
78	AGAAAUG	CUGAUGAGGCCGAAAGGCCGAA	AACAGAG
83	UCCACAG	CUGAUGAGGCCGAAAGGCCGAA	AAUGGAA
93	AUCCUAU	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAC
93	AUCCUAU	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAC
93	AUCCUAU	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAC
96	AUGAUCC	CUGAUGAGGCCGAAAGGCCGAA	AUUAGUC
96	AUGAUCC	CUGAUGAGGCCGAAAGGCCGAA	AUUAGUC
101	UAAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCUAU
104	UGCJAAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAUCC
104	UGCJAAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAUCC
106	GAUGCUA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAU

107	AGAUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGA
107	AGAUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGA
108	CAGAUGC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUG
108	CAGAUGC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUG
131	AAGCCUG	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAU
142	UGUAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAGC
142	UGUAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAGC
143	AUGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
143	AUGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
143	AUGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
143	AUGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
144	GAUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAA
144	GAUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAA
144	GAUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAA
147	AGAGAUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAA
153	AGAAACA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGUA
165	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAGA
165	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAGA
165	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAGA
166	CUCACAA	CUGAUGAGGCCGAAAGGCCGAA	AAUCGAG
167	GCUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCCA
167	GCUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCCA
167	GCUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCCA
168	GGCUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUCG
168	GGCUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUCG
197	UAGAGCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGAGC
202	GAAUCUA	CUGAUGAGGCCGAAAGGCCGAA	AGCCAAU
208	AGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUCUAGA
216	AUGGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGG
217	GAUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAG
217	GAUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAG
217	GAUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAG
218	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
218	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
218	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
218	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
224	AGAACAU	CUGAUGAGGCCGAAAGGCCGAA	AUGGGGA
224	AGAACAU	CUGAUGAGGCCGAAAGGCCGAA	AUGGGGA
230	CUUUGGA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGA
232	UGCTUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAACAU
232	UGCTUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAACAU
232	UGCTUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAACAU
241	AGCTUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCTUU
241	AGCTUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCTUU
241	AGCTUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCTUU
249	CAAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGCTUCA
264	AUCAACU	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUG
287	ACUUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGUG
295	ACAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUUGAG

295	ACAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUUGAG
296	GACAUGG	CUGAUGAGGCCGAAAGGCCGAA	AACUUGA
297	GGACAUG	CUGAUGAGGCCGAAAGGCCGAA	AAACUUG
297	GGACAUG	CUGAUGAGGCCGAAAGGCCGAA	AAACUUG
314	AGAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAGCC
314	AGAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAGCC
315	AAGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAGC
315	AAGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAGC
317	CAAAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUGA
318	ACAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAUG
318	ACAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAUG
320	GCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAA
320	GCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAA
322	CAGCACA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAG
322	CAGCACA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAG
323	GCAGCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAA
336	GAAAGAC	CUGAUGAGGCCGAAAGGCCGAA	AAUCAGC
341	CUUGUGA	CUGAUGAGGCCGAAAGGCCGAA	AGACGAA
341	CUUGUGA	CUGAUGAGGCCGAAAGGCCGAA	AGACGAA
342	ACUUGUG	CUGAUGAGGCCGAAAGGCCGAA	AAGACGA
343	CACUUGU	CUGAUGAGGCCGAAAGGCCGAA	AAAGACG
343	CACUUGU	CUGAUGAGGCCGAAAGGCCGAA	AAAGACG
352	AUCUGAA	CUGAUGAGGCCGAAAGGCCGAA	ACACUUG
355	AACAUCU	CUGAUGAGGCCGAAAGGCCGAA	AAGACAC
382	UUUCACU	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGA
408	UAACGGC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGC
414	GAGUUGU	CUGAUGAGGCCGAAAGGCCGAA	ACGGCAA
414	GAGUUGU	CUGAUGAGGCCGAAAGGCCGAA	ACGGCAA
421	AUGAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUA
426	UCUUCAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGAG
439	GUCUUCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCAUC
452	GCCAGUA	CUGAUGAGGCCGAAAGGCCGAA	AUUCGGU
454	UUGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUIUCG
484	AAUGACA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAC
484	AAUGACA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAC
488	CAGCAAU	CUGAUGAGGCCGAAAGGCCGAA	ACAGACA
503	ACACUUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCC
503	ACACUUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCC
520	GUUCUUA	CUGAUGAGGCCGAAAGGCCGAA	ACUCGGG
535	GUCAUAU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCG
536	UGUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCC
538	GUUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAGU
553	AAGAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGU
553	AAGAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGU
556	GAUAAGA	CUGAUGAGGCCGAAAGGCCGAA	AGUAGGU
556	GAUAAGA	CUGAUGAGGCCGAAAGGCCGAA	AGUAGGU
560	GGAUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGAGAGU
561	AGGAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAG
561	AGGAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAG

561	AGGAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAG
566	GGCCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAUAA
566	GGCCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAUAA
581	GGUCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGGACCA
583	CCGGUCU	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAC
583	CCGGUCU	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAC
598	ACAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUGCC
608	UUUGAAC	CUGAUGAGGCCGAAAGGCCGAA	ACACAGC
611	UCUUUUG	CUGAUGAGGCCGAAAGGCCGAA	ACGACAC
611	UCUUUUG	CUGAUGAGGCCGAAAGGCCGAA	ACGACAC
612	UUCUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACGACA
641	AGUGUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUUCAU
649	UAAAGCC	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUU
649	UAAAGCC	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUU
655	CUUUACU	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAA
656	ACUUUAC	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
659	ACAACUU	CUGAUGAGGCCGAAAGGCCGAA	ACUAAAG
664	GAUGGAC	CUGAUGAGGCCGAAAGGCCGAA	ACUUUAC
667	UUUGAUG	CUGAUGAGGCCGAAAGGCCGAA	ACAACUU
671	CAGCUUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGACA
682	GGUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCAGC
682	GGUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCAGC
682	GGUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCAGC
683	GGGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUCAG
683	GGGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUCAG
685	GGGGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUC
685	GGGGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUC
687	UUGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAG
698	ACUCAGU	CUGAUGAGGCCGAAAGGCCGAA	AUGUUGG
698	ACUCAGU	CUGAUGAGGCCGAAAGGCCGAA	AUGUUGG
718	GUCUGCA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGUU
718	GUCUGCA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGUU
729	AUCCUUU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUCU
729	AUCCUUU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUCU
729	AUCCUUU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUCU
737	AGCAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUCCUUU
737	AGCAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUCCUUU
737	AGCAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUCCUUU
745	GGAAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGU
745	GGAAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGU
759	UUUGGGA	CUGAUGAGGCCGAAAGGCCGAA	ACCCCCG
759	UUUGGGA	CUGAUGAGGCCGAAAGGCCGAA	ACCCCCG
759	UUUGGGA	CUGAUGAGGCCGAAAGGCCGAA	ACCCCCG
760	CUUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AACCCCC
760	CUUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AACCCCC
760	CUUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AACCCCC
761	GCUUUGG	CUGAUGAGGCCGAAAGGCCGAA	AAACCCC
771	GAGAAGC	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUU
771	GAGAAGC	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUU

776	ACCAAGA	CUGAUGAGGCCCGAAAGGCCGAA	AAGCGAG
776	ACCAAGA	CUGAUGAGGCCCGAAAGGCCGAA	AAGCGAG
778	CAACCAA	CUGAUGAGGCCCGAAAGGCCGAA	AGAAGCG
784	AUUUUC	CUGAUGAGGCCCGAAAGGCCGAA	ACCAAGA
803	UGCCAGG	CUGAUGAGGCCCGAAAGGCCGAA	AAUUCUC
803	UGCCAGG	CUGAUGAGGCCCGAAAGGCCGAA	AAUUCUC
803	UGCCAGG	CUGAUGAGGCCCGAAAGGCCGAA	AAUUCUC
812	UCGUUU	CUGAUGAGGCCCGAAAGGCCGAA	AUGCCAG
812	UCGUUU	CUGAUGAGGCCCGAAAGGCCGAA	AUGCCAG
816	AUUGUCG	CUGAUGAGGCCCGAAAGGCCGAA	AUUGAUG
816	AUUGUCG	CUGAUGAGGCCCGAAAGGCCGAA	AUUGAUG
824	CCUGGGA	CUGAUGAGGCCCGAAAGGCCGAA	AUUGUCG
825	UCCUGGG	CUGAUGAGGCCCGAAAGGCCGAA	AAUUGUC
826	AUCCUGG	CUGAUGAGGCCCGAAAGGCCGAA	AAAUUGU
834	GAUUCAG	CUGAUGAGGCCCGAAAGGCCGAA	AUCCUGG
841	CAAUUC	CUGAUGAGGCCCGAAAGGCCGAA	AUUCAGG
841	CAAUUC	CUGAUGAGGCCCGAAAGGCCGAA	AUUCAGG
850	AAUGGUG	CUGAUGAGGCCCGAAAGGCCGAA	ACAAUUC
869	UGAAUUC	CUGAUGAGGCCCGAAAGGCCGAA	AGUUGGC
869	UGAAUUC	CUGAUGAGGCCCGAAAGGCCGAA	AGUUGGC
869	UGAAUUC	CUGAUGAGGCCCGAAAGGCCGAA	AGUUGGC
873	GUUUUGA	CUGAUGAGGCCCGAAAGGCCGAA	AUCUAGU
873	GUUUUGA	CUGAUGAGGCCCGAAAGGCCGAA	AUCUAGU
874	CGUUUUG	CUGAUGAGGCCCGAAAGGCCGAA	AAUCUAG
875	UCGUUUU	CUGAUGAGGCCCGAAAGGCCGAA	AAAUUCU
885	UGGUUUC	CUGAUGAGGCCCGAAAGGCCGAA	AGUCGUA
899	GACACUU	CUGAUGAGGCCCGAAAGGCCGAA	AUGGUGU
899	GACACUU	CUGAUGAGGCCCGAAAGGCCGAA	AUGGUGU
906	UUAAUGA	CUGAUGAGGCCCGAAAGGCCGAA	ACACUUA
906	UUAAUGA	CUGAUGAGGCCCGAAAGGCCGAA	ACACUUA
908	AUUUAAU	CUGAUGAGGCCCGAAAGGCCGAA	AGACACU
911	CAUUUUU	CUGAUGAGGCCCGAAAGGCCGAA	AUGAGAC
916	AUCUCCA	CUGAUGAGGCCCGAAAGGCCGAA	AUUUAAU
916	AUCUCCA	CUGAUGAGGCCCGAAAGGCCGAA	AUUUAAU
943	CCAGGUG	CUGAUGAGGCCCGAAAGGCCGAA	AGUCCUC
944	CCCAGGU	CUGAUGAGGCCCGAAAGGCCGAA	AAGUCCU
1001	CUGCCCC	CUGAUGAGGCCCGAAAGGCCGAA	AAGAGCA
1034	CGAUGAC	CUGAUGAGGCCCGAAAGGCCGAA	ACGACUG
1037	CAACGAU	CUGAUGAGGCCCGAAAGGCCGAA	ACGACGA
1043	UGAUGAC	CUGAUGAGGCCCGAAAGGCCGAA	ACGAUGA
1046	UGAUGAU	CUGAUGAGGCCCGAAAGGCCGAA	ACAACGA
1049	AUUUGAU	CUGAUGAGGCCCGAAAGGCCGAA	AUGACAA
1060	CUUACAG	CUGAUGAGGCCCGAAAGGCCGAA	AGCAUUU
1060	CUUACAG	CUGAUGAGGCCCGAAAGGCCGAA	AGCAUUU
1060	CUUACAG	CUGAUGAGGCCCGAAAGGCCGAA	AGCAUUU
1060	CUUACAG	CUGAUGAGGCCCGAAAGGCCGAA	AGCAUUU
1061	GCUUACA	CUGAUGAGGCCCGAAAGGCCGAA	AAGCAUU
1080	CUUCUGA	CUGAUGAGGCCCGAAAGGCCGAA	ACAGCUU
1080	CUUCUGA	CUGAUGAGGCCCGAAAGGCCGAA	ACAGCUU

1081	UCUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AACAGCU
1121	CGAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCUGU
1121	CGAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCUGU
1121	CGAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCUGU
1122	CCGAAGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUG
1126	AGGCCCG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAA
1127	CAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAA
1127	CAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAA
1144	UUCAGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUC
1144	UUCAGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUC
1145	GUUCAGC	CUGAUGAGGCCGAAAGGCCGAA	AAUGCUU
1160	AAAGGAA	CUGAUGAGGCCGAAAGGCCGAA	ACGGUCU
1162	CUAAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGACGGU
1163	ACUAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAGACGG
1167	AAGAACT	CUGAUGAGGCCGAAAGGCCGAA	AAGGAAG
1177	AUGGACA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAA
1181	CCACAUG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAGA
1181	CCACAUG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAGA
1192	UACCAUG	CUGAUGAGGCCGAAAGGCCGAA	AUCCAC
1199	CACAUAA	CUGAUGAGGCCGAAAGGCCGAA	ACCAUGU
1201	GCCACAU	CUGAUGAGGCCGAAAGGCCGAA	AUACCAU
1210	ACCUCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCCACA
1210	ACCUCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCCACA
1223	AAAGAAA	CUGAUGAGGCCGAAAGGCCGAA	AUUGUAC
1225	UGAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AGAUUGU
1225	UGAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AGAUUGU
1226	CUGAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAUUG
1227	GCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUU
1227	GCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUU
1227	GCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUU
1229	GUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGAAAGA
1230	GGUGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGAAAG
1252	UGUCCGA	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCAG
1274	UUAACTC	CUGAUGAGGCCGAAAGGCCGAA	AUCTUGU
1310	GGAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCUCA
1312	AUGGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCCU
1314	UGAUGGA	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUC
1316	CCUGAUG	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAA
1320	GCUUCCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGAAA
1320	GCUUCCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGAAA
1339	CCCAGCA	CUGAUGAGGCCGAAAGGCCGAA	ACTUUGCC
1355	AUCAAGC	CUGAUGAGGCCGAAAGGCCGAA	AUCAAG
1437	UUUUUCU	CUGAUGAGGCCGAAAGGCCGAA	AUACCAC
1437	UUUUUCU	CUGAUGAGGCCGAAAGGCCGAA	AUACCAC
1475	GCAGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACUAGGC
1477	UUGCAGU	CUGAUGAGGCCGAAAGGCCGAA	AGACUAG
1487	ACAUUUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGCA
1491	CAUGACA	CUGAUGAGGCCGAAAGGCCGAA	AUCAAGU
1491	CAUGACA	CUGAUGAGGCCGAAAGGCCGAA	AUCAAGU

1505	AGACACC	CUGAUGAGGCCGAAAGGCCGAA	ACCAAAC
1530	CUUCAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGGCA
1531	UCUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGGC
1532	CUCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGGG
1532	CUCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGGG
1644	ACAUCCC	CUGAUGAGGCCGAAAGGCCGAA	ACCAUAG
1652	CCGUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCCC
1652	CCGUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCCC
1670	UAAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUUA
1674	UAUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AUUUUAU
1676	UUUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUUA
1677	UUUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1677	UUUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1694	UUUGCUC	CUGAUGAGGCCGAAAGGCCGAA	AUACUCU

Table BVI: Human B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
16	GAAAGCU U UGCUUCU	271	UAGUAGU A UUUUGGC
17	AAAGCUU U GCUUCUC	273	GUAGUAU U UUGGCAG
21	CUUUGCU U CUCUGCU	274	UAGUAUU U UGGCAGG
22	UUUGCUU C UCUGCUG	275	AGUAUUU U GGCAGGA
24	UGCUUCU C UGCUGCU	294	GAAAACU U GGUUCUG
34	CUGCUGU A ACAGGGA	298	ACUUGGU U CUGAUG
44	AGGGACU A GCACAGA	299	CUUGGUU C UGAAUGA
70	GUGGGGU C AUUUCCA	310	AUGAGGU A UACUUAG
73	GGGUCAU U UCCAGAU	312	GAGGUAU A CUUAGGC
74	GGUCAUU U CCAGAU	315	GUUAUCU U AGGCAAA
75	GUCAUUU C CAGAU	316	UAUACU A GGCAAAG
81	UCCAGAU A UUAGGUC	330	GAGAAU U UGACAGU
83	CAGAUU U AGGUCAC	331	AGAAAU U GACAGUG
84	AGAUUU A GGUCACA	340	ACAGUGU U CAUUGCA
88	AUUAGGU C ACAGCAG	341	CAGUGUU C AUUCCAA
113	AAUGGAU C CCCAGUG	344	UGUUCAU U CCAAGUA
125	GUGCACU A UGGGACU	345	GUUCAUU C CAAGU
137	ACUGAGU A ACAUUCU	351	UCCAAGU A UAUGGGC
142	GUAACAU U CUCUUUG	353	CAAGU A UGGGCCG
143	UAACAUU C UCUUUGU	368	CACAAGU U UUGAUUC
145	ACAUUCU C UUUGUGA	369	ACAAGUU U UGAUUCG
147	AUUCUCU U UGUGAUG	370	CAAGUUU U GAUUCGG
148	UUCUCUU U GUGAUGG	374	UUUUGAU U CGGACAG
159	AUGGCCU U CCUGCUC	375	UUUGAUU C GGACAGU
160	UGGCCUU C CUGCUCU	383	GGACAGU U GGACCCU
166	UCCUGCU C UCUGGUG	397	UGAGACU U CACAAUC
168	CUGCUCU C UGGUGCU	398	GAGACUU C ACAUUCU
179	UGCUGCU C CUCUGAA	404	UCACAAU C UUCAGAU
182	UGCUCU C UGAAGAU	406	ACAAUCU U CAGAUCA
190	UGAAGAU U CAAGCUU	407	CAAUCUU C AGAUCAA
191	GAAGAUU C AAGCUUA	412	UUCAGAU C AAGGACA
197	UCAAGCU U AUUCAA	426	AAGGGCU U GUUCAA
198	CAAGCUU A UUUCAA	429	GGCUUGU A UCAAUGU
200	AGCUU AU UCAAUGA	431	CUUGU AU C AAUGU
201	GCUU AUU U CAAUGAG	437	UCAUGU A UCAUCCA
202	CUU AUUU C AAUGAGA	439	AAUGU AU C AUCCAUC
231	UGCCAAU U UGCAAAC	442	GUU CAU C CAUCACA
232	GCCAAU U GCAAACU	446	CAUCCA C ACAAAA
240	GCAAACU C UCAAAC	469	GAAUGAU U CGCAUCC
242	AAACUCU C AAAACCA	470	AAUGAUU C GCAUCCA
265	GUGAGCU A GUAGU AU	475	UUCGCAU C CACCAGA
268	AGCUAGU A GUU AUU	488	GAUGAAU U CUGAACT

489	AUGAAU C UGAACUG	721	UGUCUGU U UCAUCC
498	GAACUGU C AGUGCUU	722	GUCUGUU U CAUCCCC
505	CAGUGCU U GCUAACU	723	UCUGUUU C AUUCCCU
509	GCUUGCU A ACTUCAG	726	GUUUCAU U CCCUGAU
513	GCUAACU U CAGUCA	727	UUUCAU C CCUGAUG
514	CUAACU C AGUCAAC	736	CUGAUGU U ACGAGCA
518	CUUCAGU C AACCUGA	737	UGAUGUU A CGAGCAA
529	CUGAAAU A GUACCAA	746	GAGCAAU A UGACCAU
532	AAAUAGU A CCAUUU	754	UGACCAU C UUCUGUA
538	UACCAAU U UCUAAUA	756	ACCAUCU U CUGUAUU
539	ACCAAUU U CUAAUAU	757	CCAUCUU C UGUUUC
540	CCAAUUU C UAAUAUA	761	CUUCUGU A UUCUGGA
542	AAUUUCU A AUUAAC	763	UCUGUAU U CUGGAAA
545	UUCUAAU A UAACAGA	764	CUGUAUU C UGGAAAC
547	CUAAUAU A ACAGAAA	787	CGCGGCU U UUAUCUU
561	AAUGUGU A CAUAAU	788	GCGGCUU U UAUCUUC
565	UGUACAU A AAUUGA	789	CGGCUUU U AUCUUA
569	CAUAAU U UGACCUG	790	GGCUUUU A UCUUCAC
570	AUAAAUU U GACCUGC	792	CUUUUAU C UUCACCU
579	ACCUGCU C AUCUAUA	794	UUUAUCU U CACCUUU
582	UGCUCAU C UAUACAC	795	UUAUCUU C ACCUUC
584	CUCAUCU A UACACGG	800	UUCACCU U UCUCUAU
586	CAUCUAU A CACGGUU	801	UCACCUU U CUCUAUA
593	ACACGGU U ACCCAGA	802	CACCUUU C UCUAUAG
594	CACGGUU A CCCAGAA	804	CCUUUCU C UAUAGAG
605	AGAACCU A AGAAGAU	806	UUUCUCU A UAGAGCU
619	UGAGUGU U UUGCUAA	808	UCUCUAU A GAGCUUG
620	GAGUGUU U UGCUAAG	814	UAGAGCU U GAGGACC
621	AGUGUUU U GCUAAGA	824	GGACCCU C AGCCUCC
625	UUUUGCU A AGAACCA	830	UCAGCCU C CCCCAGA
638	CAAGAAU U CAACUAU	844	ACCACAU U CCUUGGA
639	AAGAAUU C AACUAUC	845	CCACAUU C CUUGGAU
644	UUCAACU A UCGAGUA	848	CAUCCU U GGAUUAC
646	CAACUAU C GAGUAUG	853	CUJGGAU U ACAGCUG
651	AUCGAGU A UGAUGGU	854	UUGGAUU A CAGCUGU
659	UGAUGGU A UUAUGCA	862	CAGCUGU A CUUCCAA
661	AUGGUUU U AUGCAGA	865	CUGUACU U CCAACAG
662	UGGUUUU A UGCAGAA	866	UGUACUU C CAACAGU
672	CAGAAU C UCAAGAU	874	CAACAGU U AUUAUAU
674	GAAAUUCU C AAGAUAA	875	AACAGUU A UUAUAUG
680	UCAAGAU A AUGUCAC	877	CAGUUAU U AUAUGUG
685	AUAUAUGU C ACAGAAC	878	AGUUAUU A UAUGUGU
696	GAACUGU A CGACGUU	880	UUAUUUAU A UGUGUGA
703	ACGACGU U UCCAUCA	892	UGAUGGU U UUCUGUC
704	CGACGUU U CCAUCAG	893	GAUGGUU U UCUGUCU
705	GACGUUU C CAUCAGC	894	AUGGUUU U CUGUCUA
709	UUUCCAU C AGCUUGU	895	UGGUUUU C UGUCUAA
714	AUCAGCU U GUCUGUU	899	UUUCUGU C UAAUUCU
717	AGCUUGU C UGUUUA	901	UCUGUCU A AUUCUAU

904	GUCUAAU	U	CUAUGGA
905	UCUAAUU	C	UAUGGAA
907	UAAUUCU	A	UGGAAAU
935	GCGGCCU	C	GCAACUC
942	CGCAACU	C	UUAUAAA
944	CAACUCU	U	AUAAAUG
945	AACUCUU	A	UAAAUGU
947	CUCUUAU	A	AAUGUGG
1009	AAAAAAU	C	CAUAUAC
1013	AAUCCAU	A	UACCUGA
1015	UCCAUAU	A	CCUGAAA
1026	GAAAGAU	C	UGAUGAA
1045	AGCGUGU	U	UUUAAAA
1046	GCGUGUU	U	UUAAAAG
1047	CGUGUUU	U	UAAAAGU
1048	GUGUUUU	U	AAAAGUU
1049	UGUUUUU	A	AAAGUUC
1055	UAAAAGU	U	CGAAGAC
1056	AAAAGUU	C	GAAGACA
1065	AAGACAU	C	UUCAUGC
1067	GACAUCU	U	CAUGCGA
1068	ACAUCUU	C	AUGCGAC
1085	AAGUGAU	A	CAUGUUU
1091	UACAUGU	U	UUUAAUU
1092	ACAUGUU	U	UUAUUUA
1093	CAUGUUU	U	UAAUUAA
1094	AUGUUUU	U	AAUUAAA
1095	UGUUUUU	A	AUUAAAG
1098	UUUUAAU	U	AAAGAGU
1099	UUUAAUU	A	AAGAGUA

Table BVII: Human B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
16	AGAAGCA CUGAUGAGGCCGAAAGGCCGAA AGCUUUC
17	GAGAAGC CUGAUGAGGCCGAAAGGCCGAA AAGCUUU
21	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCAAAG
22	CAGCAGA CUGAUGAGGCCGAAAGGCCGAA AAGCAAA
24	AGCAGCA CUGAUGAGGCCGAAAGGCCGAA AGAAGCA
34	UCCUGU CUGAUGAGGCCGAAAGGCCGAA ACAGCAG
44	UCUGUGC CUGAUGAGGCCGAAAGGCCGAA AGUCCCU
70	UGGAAAU CUGAUGAGGCCGAAAGGCCGAA ACCCCAC
73	AUCUGGA CUGAUGAGGCCGAAAGGCCGAA AUGACCC
74	UAUCUGG CUGAUGAGGCCGAAAGGCCGAA AAUGACC
75	AUAUCUG CUGAUGAGGCCGAAAGGCCGAA AAAUGAC
81	GACCUAA CUGAUGAGGCCGAAAGGCCGAA AUCUGGA
83	GUGACCU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
84	UGUGACC CUGAUGAGGCCGAAAGGCCGAA AAUAUCU
88	CUGCUGU CUGAUGAGGCCGAAAGGCCGAA ACCUAAU
113	CACUGGG CUGAUGAGGCCGAAAGGCCGAA AUCCAUU
125	AGUCCCA CUGAUGAGGCCGAAAGGCCGAA AGUGCAC
137	AGAAUGU CUGAUGAGGCCGAAAGGCCGAA ACUCAGU
142	CAAAGAG CUGAUGAGGCCGAAAGGCCGAA AUGUUAC
143	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAUGUUA
145	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AGAAUGU
147	CAUCACA CUGAUGAGGCCGAAAGGCCGAA AGAGAAU
148	CCAUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAGAA
159	GAGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCCAU
160	AGAGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCCA
166	CACCAGA CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
168	AGCACCA CUGAUGAGGCCGAAAGGCCGAA AGAGCAG
179	UUCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGCA
182	AUCUJCA CUGAUGAGGCCGAAAGGCCGAA AGGAGCA
190	AAGCUUG CUGAUGAGGCCGAAAGGCCGAA AUCUJCA
191	UAAGCUU CUGAUGAGGCCGAAAGGCCGAA AAUCUUC
197	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGA
198	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
200	UCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
201	CUCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
202	UCUCAUU CUGAUGAGGCCGAAAGGCCGAA AAAUAAG
231	GUUUGCA CUGAUGAGGCCGAAAGGCCGAA AUUGGCA
232	AGUUUGC CUGAUGAGGCCGAAAGGCCGAA AAUUGGC
240	GUUUUGA CUGAUGAGGCCGAAAGGCCGAA AGUUUGC
242	UGGUUUU CUGAUGAGGCCGAAAGGCCGAA AGAGUUU
265	AUACUAC CUGAUGAGGCCGAAAGGCCGAA AGCUCAC

268	AAAUAUC	CUGAUGAGGCCGAAAGGCCGAA	ACUAGCU
271	GCCAAAA	CUGAUGAGGCCGAAAGGCCGAA	ACUACUA
273	CUGCCAA	CUGAUGAGGCCGAAAGGCCGAA	AUACUAC
274	CCUGCCA	CUGAUGAGGCCGAAAGGCCGAA	AAUACUA
275	UCCUGCC	CUGAUGAGGCCGAAAGGCCGAA	AAAUACU
294	CAGAAC	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUC
298	CAUUCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAAGU
299	UCAUUC	CUGAUGAGGCCGAAAGGCCGAA	AACCAAG
310	CUAAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCAU
312	GCCUAAG	CUGAUGAGGCCGAAAGGCCGAA	AUACCUC
315	UUUGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGUAUAC
316	CUUUGCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUAUA
330	ACUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUC
331	CACUGUC	CUGAUGAGGCCGAAAGGCCGAA	AAUUUCU
340	UGGAAUG	CUGAUGAGGCCGAAAGGCCGAA	ACACUGU
341	UUGGAAU	CUGAUGAGGCCGAAAGGCCGAA	AACACUG
344	UACUUGG	CUGAUGAGGCCGAAAGGCCGAA	AUGAACA
345	AUACUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAC
351	GCCCAUA	CUGAUGAGGCCGAAAGGCCGAA	ACTUGGA
353	CGGCCCA	CUGAUGAGGCCGAAAGGCCGAA	AUACUUG
368	GAAUCAA	CUGAUGAGGCCGAAAGGCCGAA	ACTUGUG
369	CGAAUCA	CUGAUGAGGCCGAAAGGCCGAA	AACUUGU
370	CCGAAUC	CUGAUGAGGCCGAAAGGCCGAA	AAACUUG
374	CUGUCCG	CUGAUGAGGCCGAAAGGCCGAA	AUCAAAA
375	ACUGUCC	CUGAUGAGGCCGAAAGGCCGAA	AAUCAAA
383	AGGGUCC	CUGAUGAGGCCGAAAGGCCGAA	ACUGUCC
397	GAUUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCA
398	AGAUGUG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUC
404	AUCUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUUGUGA
406	UGAUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGUG
407	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUUG
412	UGUCCUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAA
426	UGAUUAC	CUGAUGAGGCCGAAAGGCCGAA	AGCCCUU
429	ACAUUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAAGCC
431	AUACAUU	CUGAUGAGGCCGAAAGGCCGAA	AUACAAG
437	UGGAUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGA
439	GAUGGAU	CUGAUGAGGCCGAAAGGCCGAA	AUACAUU
442	UGUGAUG	CUGAUGAGGCCGAAAGGCCGAA	AUGAUAC
446	UUUUUGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUG
469	GGAUGCG	CUGAUGAGGCCGAAAGGCCGAA	AUCAUUC
470	UGGAUGC	CUGAUGAGGCCGAAAGGCCGAA	AAUCAUU
475	UCUGGUG	CUGAUGAGGCCGAAAGGCCGAA	AUGCGAA
488	AGUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUUCAUC
489	CAGUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAUUCAU
498	AAGCACU	CUGAUGAGGCCGAAAGGCCGAA	ACAGUUC
505	AGUUAGC	CUGAUGAGGCCGAAAGGCCGAA	AGCACUG
509	CUGAAGU	CUGAUGAGGCCGAAAGGCCGAA	AGCAAGC
513	UUGACUG	CUGAUGAGGCCGAAAGGCCGAA	AGUUAGC
514	GUUGACU	CUGAUGAGGCCGAAAGGCCGAA	AAGUUAG

518	UCAGGUU	CUGAUGAGGCCGAAAGGCCGAA	ACUGAAG
529	UUGGUAC	CUGAUGAGGCCGAAAGGCCGAA	AUUUCAG
532	AAAUUGG	CUGAUGAGGCCGAAAGGCCGAA	ACUAAUU
538	UAUUAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGUA
539	AUAUUAG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGU
540	UAUAUUA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGG
542	GUUAUUA	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUU
545	UCUGUUA	CUGAUGAGGCCGAAAGGCCGAA	AUUAGAA
547	UUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUAG
561	AUUUAUG	CUGAUGAGGCCGAAAGGCCGAA	ACACAUU
565	UCAAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUGUACA
569	CAGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAUG
570	GCAGGUC	CUGAUGAGGCCGAAAGGCCGAA	AAUUUAU
579	UAUAGAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGU
582	GUGUAUA	CUGAUGAGGCCGAAAGGCCGAA	AUGAGCA
584	CCGUGUA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAG
586	AACCGUG	CUGAUGAGGCCGAAAGGCCGAA	AUAGAUG
593	UCUGGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCGUGU
594	UUCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AACCGUG
605	AUCUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCU
619	UUAGCAA	CUGAUGAGGCCGAAAGGCCGAA	ACACUCA
620	CUUAGCA	CUGAUGAGGCCGAAAGGCCGAA	AACACUC
621	UCUUAGC	CUGAUGAGGCCGAAAGGCCGAA	AAACACU
625	UGGUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAA
638	AUAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUUG
639	GAUAGUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUU
644	UACUCGA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGAA
646	CAUACUC	CUGAUGAGGCCGAAAGGCCGAA	AUAGUUG
651	ACCAUCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCGAU
659	UGCAUAA	CUGAUGAGGCCGAAAGGCCGAA	ACCAUCA
661	UCUGCAU	CUGAUGAGGCCGAAAGGCCGAA	AUACCAU
662	UUCUGCA	CUGAUGAGGCCGAAAGGCCGAA	AAUACCA
672	AUCUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUG
674	UUAUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGAUUUC
680	GUGACAU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGA
685	GUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUAU
696	AACGUCG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUUC
703	UGAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACGUCGU
704	CUGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AACGUCG
705	GCUGAUG	CUGAUGAGGCCGAAAGGCCGAA	AAACGUC
709	ACAAGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGAAA
714	AACAGAC	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAU
717	UGAAACA	CUGAUGAGGCCGAAAGGCCGAA	ACAAGCU
721	GGAAUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGACA
722	GGGAAUG	CUGAUGAGGCCGAAAGGCCGAA	AACAGAC
723	AGGGAAU	CUGAUGAGGCCGAAAGGCCGAA	AAACAGA
726	AUCAGGG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAAC
727	CAUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAA
736	UGCUCGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAG

737	UUGCUCG	CUGAUGAGGCCGAAAGGCCGAA	AACAUCA
746	AUGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUGCUC
754	UACAGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGGUCA
756	AAUACAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGU
757	GAAUACA	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGG
761	UCCAGAA	CUGAUGAGGCCGAAAGGCCGAA	ACAGAAG
763	UUUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUACAGA
764	GUUCCA	CUGAUGAGGCCGAAAGGCCGAA	AAUACAG
787	AAGAUAA	CUGAUGAGGCCGAAAGGCCGAA	AGCCGCG
788	GAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AAGCCGC
789	UGAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCG
790	GUGAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGCC
792	AGGUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAAG
794	AAAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUAAA
795	GAAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUAA
800	AUAGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUGAA
801	UAUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUGA
802	CUAUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGGUG
804	CUCUAUA	CUGAUGAGGCCGAAAGGCCGAA	AGAAAGG
806	AGCUCUA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAA
808	CAAGCUC	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGA
814	GGUCCUC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCUA
824	GGAGGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGGUCC
830	UCUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUGA
844	UCCAAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGUGGU
845	AUCCAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUGUGG
848	GUAUCC	CUGAUGAGGCCGAAAGGCCGAA	AGGAAUG
853	CAGCUGU	CUGAUGAGGCCGAAAGGCCGAA	AUCCAAG
854	ACAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAUCCAA
862	UUGGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUG
865	CUGUUGG	CUGAUGAGGCCGAAAGGCCGAA	AGUACAG
866	ACUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AAGUACA
874	AUAUAU	CUGAUGAGGCCGAAAGGCCGAA	ACUGUUG
875	CAUAUAA	CUGAUGAGGCCGAAAGGCCGAA	AACUGUU
877	CACAUAU	CUGAUGAGGCCGAAAGGCCGAA	AUAACUG
878	ACACAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAACU
880	UCACACA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
892	GACAGAA	CUGAUGAGGCCGAAAGGCCGAA	ACCAUCA
893	AGACAGA	CUGAUGAGGCCGAAAGGCCGAA	AACCAUC
894	UAGACAG	CUGAUGAGGCCGAAAGGCCGAA	AAACCAU
895	UUAGACA	CUGAUGAGGCCGAAAGGCCGAA	AAAACCA
899	AGAAUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAGAAA
901	AUAGAAU	CUGAUGAGGCCGAAAGGCCGAA	AGACAGA
904	UCCAUAG	CUGAUGAGGCCGAAAGGCCGAA	AUUAGAC
905	UUCCAUA	CUGAUGAGGCCGAAAGGCCGAA	AAUUAGA
907	AUUUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUA
935	GAGUUGC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGC
942	UUUAUAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGCG
944	CAUUUAU	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG

945	ACAUUUA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGUU
947	CCACAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAAGAG
1009	GUUAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUU
1013	UCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUU
1015	UUUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAUGGA
1026	UUCAUCA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUC
1045	UUUUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACACGCU
1046	CUUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AACACGC
1047	ACUUUUA	CUGAUGAGGCCGAAAGGCCGAA	AAACACG
1048	AACUUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAACAC
1049	GAACUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAACA
1055	GUCUUCG	CUGAUGAGGCCGAAAGGCCGAA	ACUUUUA
1056	UGUCUUC	CUGAUGAGGCCGAAAGGCCGAA	AACUUUU
1065	GCAUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUU
1067	UCGCAUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGUC
1068	GUCGCAU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGU
1085	AAACAUG	CUGAUGAGGCCGAAAGGCCGAA	AUCACUU
1091	AAUJAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUGUA
1092	UAAUJAA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGU
1093	UUAAUUA	CUGAUGAGGCCGAAAGGCCGAA	AAACAUG
1094	UUUJAAU	CUGAUGAGGCCGAAAGGCCGAA	AAAACAU
1095	CUUUJAU	CUGAUGAGGCCGAAAGGCCGAA	AAAAACA
1098	ACUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUAAAA
1099	UACUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAAA

Table BVIII: Mouse B7-2 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
47	AcGGACU u GaACAac	194	cuUAuUU C aAUGGgA
47	aCggACU u gaAcAAC	208	acUGCaU a UCUGCcG
66	CUccUgU a gAcGUgU	210	UGCaUaU C UGCcGug
66	CUCcUgU A gAcGUGu	223	UGCCcAU U UaCAAAg
74	gAcGUGU u CcagAAc	223	UGCCcAU u UAcAaAg
83	CaGaACU U aCggaAG	224	GCCcAUU U aCAAagg
134	caAuCcU U aUCUUUG	225	ccCAUUU a CAaAggc
134	CaauccU U AUCUUug	225	CccaUUU a cAAAgGc
134	caAUCcU u AuCUUUG	242	AAaACAU a agCcUGa
134	CAaUccU U AUcUuUG	260	AGCUgGU A GUUUUUU
134	CAAucCU U AUcnuUG	260	aGCuGgU a gUAUuUU
135	aAuCcUU a UCUUUGU	263	UgGUAGU A UUUUGGC
135	aAuCcUU a UCuUugu	263	UGgUaGU a UUuUGgC
135	AaUccUU A UcUuUGU	265	GUAGUAU U UUGGCAG
135	aAUccUU a UCuUugU	265	guAGUAU u UuGGCaG
137	uCcUUaU C UUUGUGA	266	UAGUAUU U UGGCAGG
137	UccUUaU c UuUGUGA	266	uAGUaUU U UGgcAgG
137	UCCuUAU c uuUGugA	266	UAgUauU u UGGcAgg
139	cUUaUCU U UGUGAca	267	AGUAUUU U GGCAGGA
140	UUaUCUU U GUGAcaG	267	AGUaUUU U GgcAgGA
140	UUaUcuU U guGACAG	286	cAAAagU U GGUUCUG
149	UGAcAGU c UUGCUGA	286	CAAAagU U GgUUCuG
151	AcAGucU U GCUGaUC	290	AgUUGGU U CUGuAcG
151	AcaGuCU U gCUGaUC	291	gUUGGUU C UGuAcGA
158	UgCuGAU c UcAGaUg	295	GUUCuGU a CgAGcAc
158	UgCUGaU C UCaGaUG	304	GAGcacU A uUUgGGC
158	UGcUgAU c uCagaUg	307	cacUAUU u GGgCACA
158	UgCugAU c UCagAUg	323	AGAAAcU U GAuAGUG
160	CUGaUCU C aGaUGCU	343	gCCAAGU A ccUGGGC
160	cUGaUcU c AgAuGcU	343	gCCAagU a CCUgGGc
170	AUGcuGU u UcCgUgG	361	ACgAGcU U UGAcagG
171	UGCUGuU u CcgUGgA	381	cUGgACU c UacGACU
172	gCUgUuU C cgUgGAG	383	GgACUcU A CGACuUc
189	GcaaGcU u AUUUCaA	383	GGACuCU a cGaCUuC
189	gCAAGCU U AUUUCAA	389	uAcGacU u CaCAaUG
189	GCaaGCU u AuUUCaA	389	UacGACU U CACAAUg
190	CAAGCUU A UUUCAAU	390	acGACUU C ACAAUgU
190	CaAgcUU a uUUCaAU	390	ACgAcUU c acAAUgU
192	AGCUUAU U UCAAUGg	398	ACAAUGU U CAgauCA
192	aGCUUaU u UCAAUGg	398	ACAAUGU U CAGAUCA
193	GCUUAUU U CAAUGgG	398	ACaAuGU U cagAUCA
193	GcuUAuU U CaAUGGg	399	CAaUGUU C AgauCAA
194	CUUAUUU C AAUGgGA	399	CAAUgUU C AGAUCAA

399	CaAuGUU c	agAUCAa	658	CAGAUAU c	AcaagAu
399	caAUGUU c	aGAuCAA	658	CAGauAU C	ACAAGAu
399	CAaUguU c	aGAUcAa	658	CAGAuAU C	aCAAGAU
399	cAAuGuU C	aGAUcAA	658	CaGAUaU c	ACaAGAu
399	CAaugUU c	agAUcAA	666	aCAAGAU A	AUGUCAC
404	UUCAGAU C	AAGGACA	666	ACAagaU a	AUGucAC
404	UucAGaU c	aAGGACa	671	AUaAuGU C	ACAGaAc
418	aUGgGCU c	GUAugAU	671	aUAAUGU c	ACAGAAC
418	AuGGGCU c	GUAugAu	671	AUAAUGU C	ACAGAAC
418	AUggGCU c	GUaUGaU	682	gAACTUGu u	cAGUAUc
421	gGCUCgU a	UGAuugU	683	aAcUGuU c	aGuAUCu
421	ggCUCgU A	UgAuUGU	683	AAcUGuU c	agUaUcU
429	UgAuUGU u	UuAUaCA	691	aguaUcU C	CAaCAGC
429	UGAUuGU u	UUAUaCA	691	agUAUCU c	CAaCagc
431	AuUgUuU u	AUAcAAa	691	aGUaUcU C	CAACAGc
431	AUuGUuU U	AUaCAaA	701	aCaGCcU c	UcUCUHu
432	UuGUuUU A	UaCAaAA	701	acagCCU c	UCUCUuU
432	UuGUUUU a	UacaaAA	703	AGCcUcU C	UcUUUCA
432	uUGUUUU a	uAcaAAA	703	aGCcUcU c	UCUHuca
461	gAUcaAU u	AUCCucC	707	UcUCUcU U	UCAUUCc
462	AucaAUU a	uCcUCCA	707	UcUCUcU u	UcAUUCC
464	CAauUaU c	CUcCaAc	708	cUCUcUU U	CAUUCcC
467	uUAUCcU C	CAaCAGa	709	UCUcUUU C	AUUCcCg
467	UUauCcU C	CAaCAGA	709	UCUCUuU c	auuCccG
467	UUaUccU c	cAACAGA	709	UCUcUuU c	AUUCccg
467	UuAuCCU C	CaaCAGA	712	CUUucaU U	CcCgGaU
490	GAACUGU C	AGUGaUc	712	cuuUCAU U	cCCgGAU
497	CAGUGaU c	GCCAAcU	712	CuUucaU u	CcCGGaU
505	GCCAAcU U	CAGUGaA	712	cUUUCAU U	CCCgGAU
506	CcAAcUU C	AGUGAAC	712	CUUucaU u	ccCggaU
506	CCAaCUU C	aGUGaaC	713	uuUCAUU c	CCgGAUg
521	CUGAAAU A	aaACugg	713	UUUCAUU C	CCgGAUG
531	ACUGgcU c	AgAaUgU	732	GuGgcAU a	UGACcGU
539	agaaUGU A	ACAGGaA	732	GuGgcAU A	UGACCgU
550	GgAaAuU c	uGGCAuA	740	UGACCgU u	gUgUGUg
550	ggAAaUU C	UggcAUA	749	UgUGUGU U	CUGGAAA
557	cuggCAU A	AAUUGA	749	uGuGUGU U	cUggAAA
561	CAUAAAU U	UGACCUG	750	gUGUGUU C	UGGAAAC
562	AUAAAUU U	GACCUGC	750	GuGUGUU c	UggAAAAC
576	CaCgUCU A	agCAaGG	773	ugAAGaU U	UcCUcCa
585	gCAaGGU c	ACCCgaA	778	aUUUcCU c	caAACCu
597	gaAACCU A	AGAAGAU	788	AAcCUCU C	AAuuuCA
607	AaGaUgU a	uUuUCUg	798	UUUCaCU c	aAGAGuU
611	UGUaUUU u	cUgAuAa	805	CAagAGU U	UccAUcu
625	AcuAAUU C	AACUAau	805	CAagAGU U	uccAUcU
630	UUCAACU A	auGAGUA	806	AAGAGUU u	ccAUcUc
630	UUCAAcU A	AuGAGUA	811	UUUCCA U	c ucCUcaa
637	AauGAGU A	UGgUGaU	811	uUUCcaU c	UcCUcaa
656	uGCAGaU a	UcAcAAg	813	uCCAUCU c	CUcaAac

836	aGgAGAU	U	acAGCUU
836	aggaGAU	U	ACAGCUu
837	GgAGAUU	a	cAGCUUc
848	CUUCAGU	u	AcugUGg
860	UGGCCcU	C	CUcCUug
860	UggCCcU	c	CUCcuUg
878	ugCUGCU	C	AUCauUg
951	GCGGgaU	a	GuAACgC
974	AgaCuAU	c	aACCUGA
989	aGgaAcU	U	GaACCCc
1006	auUgCUU	c	aGCAAAa
1055	AAAgAGU	u	aaAAaUU
1056	AaGAgUU	a	aaAAuUG
1062	uAAAAAU	u	gcUuUgC
1092	CAGaGUU	u	CuCAGAA
1095	aGUUUcU	c	AgAaUUC
1101	UCAgAAU	u	caaAaAU
1101	ucAGAAU	U	CAAAaAU
1101	UcAgAaU	U	CaAAaAu
1111	aAaAUGU	U	cUcAgcU
1112	AaAUGUU	c	UcAgcUg
1128	UUgGAaU	u	cuACAGU
1128	UUGGAaU	u	CuaCaGU
1131	GAAuUCU	a	cAGuUgA
1131	GAauUCU	a	CAGuuGA
1141	GuUGAAU	a	aUuAAag
1144	gaaUAAU	U	AAAGAac
1145	AAuAaUU	a	aAgaACA

Table BIX: Mouse B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences		
47	GUUGUUC	CUGAUGAGGCCGAAAGGCCGAA	AGUCCGU
47	GUUGUUC	CUGAUGAGGCCGAAAGGCCGAA	AGUCCGU
66	ACACGUC	CUGAUGAGGCCGAAAGGCCGAA	ACAGGAG
66	ACACGUC	CUGAUGAGGCCGAAAGGCCGAA	ACAGGAG
74	GUUCUGG	CUGAUGAGGCCGAAAGGCCGAA	ACACGUC
83	CUUCCGU	CUGAUGAGGCCGAAAGGCCGAA	AGUUCUG
134	CAAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAUUG
134	CAAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAUUG
134	CAAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAUUG
134	CAAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAUUG
134	CAAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAUUG
135	ACAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAUU
135	ACAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAUU
135	ACAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAUU
135	ACAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAUU
137	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUAAGGA
137	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUAAGGA
137	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUAAGGA
139	UGUCACA	CUGAUGAGGCCGAAAGGCCGAA	AGAUAA
140	CUGUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGAUAA
140	CUGUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGAUAA
149	UCAGCAA	CUGAUGAGGCCGAAAGGCCGAA	ACUGUCA
151	GAUCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGACUGU
151	GAUCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGACUGU
158	CAUCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCAGCA
158	CAUCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCAGCA
158	CAUCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCAGCA
158	CAUCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCAGCA
160	AGCAUCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUCAG
160	AGCAUCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUCAG
170	CCACGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAU
171	UCCACGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGCA
172	CUCCACG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGC
189	UUGAAAU	CUGAUGAGGCCGAAAGGCCGAA	AGCUUGC
189	UUGAAAU	CUGAUGAGGCCGAAAGGCCGAA	AGCUUGC
189	UUGAAAU	CUGAUGAGGCCGAAAGGCCGAA	AGCUUGC
190	AUUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUG
190	AUUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUG
192	CCAUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUAAGCU
192	CCAUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUAAGCU
193	CCCAUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUAAGC
193	CCCAUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUAAGC
194	UCCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAG

194	UCCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAG
208	CGGCAGA	CUGAUGAGGCCGAAAGGCCGAA	AUGCAGU
210	CACGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUGCA
223	CUUUGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
223	CUUUGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
224	CCUUGU	CUGAUGAGGCCGAAAGGCCGAA	AAUGGGC
225	GCCUUUG	CUGAUGAGGCCGAAAGGCCGAA	AAAUGGG
225	GCCUUUG	CUGAUGAGGCCGAAAGGCCGAA	AAAUGGG
242	UCAGGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGUUUU
260	AAAAUAC	CUGAUGAGGCCGAAAGGCCGAA	ACCAGCU
260	AAAAUAC	CUGAUGAGGCCGAAAGGCCGAA	ACCAGCU
263	GCCAAA	CUGAUGAGGCCGAAAGGCCGAA	ACUACCA
263	GCCAAA	CUGAUGAGGCCGAAAGGCCGAA	ACUACCA
265	CUGCCAA	CUGAUGAGGCCGAAAGGCCGAA	AUACUAC
265	CUGCCAA	CUGAUGAGGCCGAAAGGCCGAA	AUACUAC
266	CCUGCCA	CUGAUGAGGCCGAAAGGCCGAA	AAUACUA
266	CCUGCCA	CUGAUGAGGCCGAAAGGCCGAA	AAUACUA
266	CCUGCCA	CUGAUGAGGCCGAAAGGCCGAA	AAUACUA
267	UCCUGCC	CUGAUGAGGCCGAAAGGCCGAA	AAAUACU
267	UCCUGCC	CUGAUGAGGCCGAAAGGCCGAA	AAAUACU
286	CAGAAC	CUGAUGAGGCCGAAAGGCCGAA	ACUUUUG
286	CAGAAC	CUGAUGAGGCCGAAAGGCCGAA	ACUUUUG
290	CGUACAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAACTU
291	UCGUACA	CUGAUGAGGCCGAAAGGCCGAA	AACCAAC
295	GUGCUCG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAAC
304	GCCCAA	CUGAUGAGGCCGAAAGGCCGAA	AGUGCUC
307	UGUGCCC	CUGAUGAGGCCGAAAGGCCGAA	AAUAGUG
323	CACUAUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCU
343	GCCCAGG	CUGAUGAGGCCGAAAGGCCGAA	ACTUUGC
343	GCCCAGG	CUGAUGAGGCCGAAAGGCCGAA	ACTUUGC
361	CCUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUCGU
381	AGUCGUA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAG
383	GAAGUCG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUCC
383	GAAGUCG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUCC
389	CAUUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGUCGUA
389	CAUUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGUCGUA
390	ACAUUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCGU
390	ACAUUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCGU
398	UGAUCUG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGU
398	UGAUCUG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGU
398	UGAUCUG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGU
399	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AACAUUG
399	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AACAUUG
399	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AACAUUG
399	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AACAUUG
399	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AACAUUG
399	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AACAUUG
404	UGUCCUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAA

404 UGUCCUU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA
418 AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
418 AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
418 AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
421 ACAAUCA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC
421 ACAAUCA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC
429 UGUUAUA CUGAUGAGGCCGAAAGGCCGAA ACAAUCA
429 UGUUAUA CUGAUGAGGCCGAAAGGCCGAA ACAAUCA
431 UUUGUAU CUGAUGAGGCCGAAAGGCCGAA AAACAAU
431 UUUGUAU CUGAUGAGGCCGAAAGGCCGAA AAACAAU
432 UUUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA
432 UUUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA
432 UUUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA
461 GGAGGAU CUGAUGAGGCCGAAAGGCCGAA AUUGAUC
462 UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU
464 GUUGGAG CUGAUGAGGCCGAAAGGCCGAA AUAAUUG
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
490 GAUCACU CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
497 AGUUGGC CUGAUGAGGCCGAAAGGCCGAA AUCACUG
505 UUCACUG CUGAUGAGGCCGAAAGGCCGAA AGUUGGC
506 GUUCACU CUGAUGAGGCCGAAAGGCCGAA AAGUUGG
506 GUUCACU CUGAUGAGGCCGAAAGGCCGAA AAGUUGG
521 CCAGUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCAG
531 ACAUUCU CUGAUGAGGCCGAAAGGCCGAA AGCCAGU
539 UUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUCU
550 UAUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUUUCC
550 UAUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUUUCC
557 UCAAAUU CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
561 CAGGUCA CUGAUGAGGCCGAAAGGCCGAA AUUUUUG
562 GCAGGUC CUGAUGAGGCCGAAAGGCCGAA AAUUUAU
576 CCUUGCU CUGAUGAGGCCGAAAGGCCGAA AGACGUG
585 UUCGGGU CUGAUGAGGCCGAAAGGCCGAA ACCUUGC
597 AUCUUCU CUGAUGAGGCCGAAAGGCCGAA AGGUUUC
607 CAGAAA CUGAUGAGGCCGAAAGGCCGAA ACAUCUU
611 UUAUCAG CUGAUGAGGCCGAAAGGCCGAA AAUACA
625 AUUAGUU CUGAUGAGGCCGAAAGGCCGAA AAUUAGU
630 UACUCAU CUGAUGAGGCCGAAAGGCCGAA AGUUGAA
630 UACUCAU CUGAUGAGGCCGAAAGGCCGAA AGUUGAA
637 AUCACCA CUGAUGAGGCCGAAAGGCCGAA ACUCAUU
656 CUUGUGA CUGAUGAGGCCGAAAGGCCGAA AUCUGCA
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
666 GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGU
666 GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGU

671	GUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUAU
671	GUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUAU
671	GUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUAU
682	GAUACUG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUUC
683	AGAUACU	CUGAUGAGGCCGAAAGGCCGAA	AACAGUU
683	AGAUACU	CUGAUGAGGCCGAAAGGCCGAA	AACAGUU
691	GCUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUACU
691	GCUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUACU
691	GCUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUACU
701	AAAGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCUGU
701	AAAGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCUGU
703	UGAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGCU
703	UGAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGCU
707	GGAUGA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAGA
707	GGAUGA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAGA
708	GGGAAUG	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAG
709	CGGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAGA
709	CGGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAGA
709	CGGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAGA
712	AUCCGGG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAAG
712	AUCCGGG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAAG
712	AUCCGGG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAAG
712	AUCCGGG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAAG
712	AUCCGGG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAAG
713	CAUCCGG	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAA
713	CAUCCGG	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAA
732	ACGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAC
732	ACGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAC
740	CACACAC	CUGAUGAGGCCGAAAGGCCGAA	ACGGUCA
749	UUUCCAG	CUGAUGAGGCCGAAAGGCCGAA	ACACACA
749	UUUCCAG	CUGAUGAGGCCGAAAGGCCGAA	ACACACA
750	GUUCCA	CUGAUGAGGCCGAAAGGCCGAA	AACACAC
750	GUUCCA	CUGAUGAGGCCGAAAGGCCGAA	AACACAC
773	UGGAGGA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUCA
778	AGGUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAAAU
788	UGAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUU
798	AACUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGUGAAA
805	AGAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUG
805	AGAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUG
806	GAGAUUG	CUGAUGAGGCCGAAAGGCCGAA	AACUCUU
811	UUGAGGA	CUGAUGAGGCCGAAAGGCCGAA	AUGGAAA
811	UUGAGGA	CUGAUGAGGCCGAAAGGCCGAA	AUGGAAA
813	GUUUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGA
836	AAGCUGU	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCU
836	AAGCUGU	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCU
837	GAAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAUCUCC
848	CCACAGU	CUGAUGAGGCCGAAAGGCCGAA	ACUGAAG
860	CAAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGCCA
860	CAAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGCCA

878	CAAUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
951	GCGUAC	CUGAUGAGGCCGAAAGGCCGAA	AUCCCGC
974	UCAGGUU	CUGAUGAGGCCGAAAGGCCGAA	AUAGUCU
989	GGGUUC	CUGAUGAGGCCGAAAGGCCGAA	AGUCCU
1006	UUUUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGCAAU
1055	AAUUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUU
1056	CAUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACUCUU
1062	GCAAAGC	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUA
1092	UUCUGAG	CUGAUGAGGCCGAAAGGCCGAA	AACUCUG
1095	GAAUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGAAACU
1101	AUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
1101	AUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
1101	AUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
1111	AGCUGAG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUU
1112	CAGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AACAUUU
1128	ACUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAA
1128	ACUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAA
1131	UCAACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUC
1131	UCAACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUC
1141	CUUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AUUCAAC
1144	GUUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUUUC
1145	UGUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUU

Table BX: Human CD40 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
9	CCUCGCU C GGGCGCC	440	UUGGGGU C AAGCAGA
24	CAGUGGU C CUGCCGC	449	AGCAGAU U GCUACAG
37	GCCUGGU C UCACCUC	453	GAUUGCU A CAGGGGU
39	CUGGUCU C ACCUCGC	461	CAGGGGU U UCUGAUA
44	CUCACCU C GCCAUGG	462	AGGGGUU U CUGAUAC
53	CCAUGGU U CGUCUGC	463	GGGGUUU C UGAUACC
54	CAUGGUU C GUCUGCC	468	UUCUGAU A CCAUCUG
57	GGUUCGU C UGCCUCU	473	AUACCAU C UGCGAGC
63	UCUGCCU C UGCAGUG	491	GCCCAGU C GGUUCU
74	AGUGCGU C CUCUGGG	496	GUCGGCU U CUUCUCC
77	GCGUCCU C UGGGGCU	497	UCGGCUU C UUCUCCA
88	GGCUGCU U GCUGACC	499	GGCUUCU U CUCCA AU
101	CCGCUGU C CAUCCAG	500	GCUUCU C UCCAAUG
105	UGUCCAU C CAGAACC	502	UUCUUCU C CAAUGUG
139	AAACAGU A CCUAAUA	511	AAUGUGU C AUCUGCU
143	AGUACCU A AUAAACA	514	GUGUCAU C UGCUUUC
146	ACCUAAU A AACAGUC	519	AUCUGCU U UCGAAAA
153	AAACAGU C AGUGCUG	520	UCUGCUU U CGAAAAA
162	GUGCUGU U CUUUGUG	521	CUGCUUU C GAAAAAU
163	UGCUGUU C UUUGUGC	531	AAAAGU C ACCCUUG
165	CUGUUCU U UGUGCCA	537	UCACCCU U GGACAAG
166	UGUUCUU U GUGCCAG	566	ACCUGGU U GUGCAAC
208	ACAGAGU U CACUGAA	599	CUGAUGU U GUCUGUG
209	CAGAGUU C ACUGAAA	602	AUGUUGU C UGUGGUC
227	AAUGCCU U CCUUGCG	609	CUGUGGU C CCCAGGA
228	AUGCCUU C CUUGCGG	618	CCAGGAU C GGCUGAG
231	CCUUCCU U GCGGUGA	641	UGGUGAU C CCCAUCA
247	AGCGAAU U CCUAGAC	647	UCCCCAU C AUCUUCG
248	GCGAAUU C CUAGACA	650	CCAUCAU C UUCGGGA
251	AAUUCCU A GACACCU	652	AUCAUCU U CGGGAUC
292	CACAAAU A CUGCGAC	653	UCAUCUU C GGGAUCC
308	CCAACCU A GGGCUUC	659	UCGGGAU C CUGUUUG
314	UAGGGCU U CGGGUCC	664	AUCCUGU U UGCCAUC
315	AGGGCUU C GGGUCCA	665	UCCUGUU U GCCAUCC
320	UUCGGGU C CAGCAGA	671	UUGCCAU C CUCUUGG
337	GGCACCU C AGAAACA	674	CCAUCCU C UUGGUGC
353	ACACCAU C UGCACCU	676	AUCCUCU U GGUGCUG
381	GCACUGU A CGAGUGA	686	UGCUGGU C UUAUCA
407	GCUGUGU C CUGCACC	688	CUGGUCU U UAUCAAA
418	CACCGCU C AUGCUCG	689	UGGUCUU U AUCAAAA
424	UCAUGCU C GCCCGGC	690	GGUCUUU A UCAAAAA
433	CCCGGCU U UGGGGUC	692	UCUUUAU C AAAAAGG
434	CCGGCUU U GGGGUCA	720	AACCAAU A AGGCCCC

755 AGGAGAU C AAUUUUC
759 GAUCAAU U UUCCCGA
760 AUCAAUU U UCCCGAC
761 UCAAUUU U CCCGACG
762 CAAUUUU C CCGACGA
771 CGACGAU C UUCCUGG
773 ACGAUCU U CCUGGCU
774 CGAUCUU C CUGGCUC
781 CCUGGCU C CAACACU
795 UGCUGCU C CAGUGCA
810 GGAGACU U UACAUGG
811 GAGACUU U ACAUGGA
812 AGACUUU A CAUGGAU
830 AACCGGU C ACCCAGG
855 AGAGAGU C GCAUCUC
860 GUCGCAU C UCAGUGC
862 CGCAUCU C AGUGCAG
927 AGGCAGU U GGCCAGA
981 GGGAGCU A UGCCCAG
990 GCCCAGU C AGUGCCA

Table BXL: Human CD40 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
9	GGCGCCC CUGAUGAGGCCCGAAAGGCCGAA AGCGAGG
24	GCGGCAG CUGAUGAGGCCCGAAAGGCCGAA ACCACUG
37	GAGGUGA CUGAUGAGGCCCGAAAGGCCGAA ACCAGGC
39	GCGAGGU CUGAUGAGGCCCGAAAGGCCGAA AGACCAG
44	CCAUGGC CUGAUGAGGCCCGAAAGGCCGAA AGGUGAG
53	GCAGACG CUGAUGAGGCCCGAAAGGCCGAA ACCAUGG
54	GGCAGAC CUGAUGAGGCCCGAAAGGCCGAA AACC AUG
57	AGAGGCA CUGAUGAGGCCCGAAAGGCCGAA ¹⁸ ACGAACC
63	CACUGCA CUGAUGAGGCCCGAAAGGCCGAA AGGCAGA
74	CCCAGAG CUGAUGAGGCCCGAAAGGCCGAA ACGCACU
77	AGCCCCA CUGAUGAGGCCCGAAAGGCCGAA AGGACGC
88	GGUCAGC CUGAUGAGGCCCGAAAGGCCGAA AGCAGCC
101	CUGGAUG CUGAUGAGGCCCGAAAGGCCGAA ACAGCGG
105	GGUUCUG CUGAUGAGGCCCGAAAGGCCGAA AUGGACA
139	UAUUAGG CUGAUGAGGCCCGAAAGGCCGAA ACUGUUU
143	UGUUUAU CUGAUGAGGCCCGAAAGGCCGAA AGGUACU
146	GACUGUU CUGAUGAGGCCCGAAAGGCCGAA AUUAGGU
153	CAGCACTU CUGAUGAGGCCCGAAAGGCCGAA ACUGUUU
162	CACAAAG CUGAUGAGGCCCGAAAGGCCGAA ACAGCAC
163	GCACAAA CUGAUGAGGCCCGAAAGGCCGAA AACAGCA
165	UGGCACA CUGAUGAGGCCCGAAAGGCCGAA AGAACAG
166	CUGGCAC CUGAUGAGGCCCGAAAGGCCGAA AAGAACA
208	UUCAGUG CUGAUGAGGCCCGAAAGGCCGAA ACUCUGU
209	UUUCAGU CUGAUGAGGCCCGAAAGGCCGAA AACUCUG
227	CGCAAGG CUGAUGAGGCCCGAAAGGCCGAA AGGCAUU
228	CCGCAAG CUGAUGAGGCCCGAAAGGCCGAA AAGGCAU
231	UCACCGC CUGAUGAGGCCCGAAAGGCCGAA AGGAAGG
247	GUCUAGG CUGAUGAGGCCCGAAAGGCCGAA AUUCGCU
248	UGUCUAG CUGAUGAGGCCCGAAAGGCCGAA AAUUCGC
251	AGGUGUC CUGAUGAGGCCCGAAAGGCCGAA AGGAAUU
292	GUCGCAG CUGAUGAGGCCCGAAAGGCCGAA AUUUGUG
308	GAAGCCC CUGAUGAGGCCCGAAAGGCCGAA AGGUUGG
314	GGACCCG CUGAUGAGGCCCGAAAGGCCGAA AGCCCTUA
315	UGGACCC CUGAUGAGGCCCGAAAGGCCGAA AAGCCCTU
320	UCUGCUG CUGAUGAGGCCCGAAAGGCCGAA ACCCGAA
337	UGUUUCU CUGAUGAGGCCCGAAAGGCCGAA AGGUGCC
353	AGGUGCA CUGAUGAGGCCCGAAAGGCCGAA AUGGUGU
381	UCACUCG CUGAUGAGGCCCGAAAGGCCGAA ACAGUGC
407	GGUGCAG CUGAUGAGGCCCGAAAGGCCGAA ACACAGC
418	CGAGCAU CUGAUGAGGCCCGAAAGGCCGAA AGCGGUG
424	GCCGGGC CUGAUGAGGCCCGAAAGGCCGAA AGCAUGA
433	GACCCCA CUGAUGAGGCCCGAAAGGCCGAA AGCCGGG
434	UGACCCC CUGAUGAGGCCCGAAAGGCCGAA AAGCCGG

440 UCUGCUU CUGAUGAGGCCGAAAGGCCGAA ACCCCAA
449 CUGUAGC CUGAUGAGGCCGAAAGGCCGAA AUCUGCU
453 ACCCCUG CUGAUGAGGCCGAAAGGCCGAA AGCAAUC
461 UAUCAGA CUGAUGAGGCCGAAAGGCCGAA ACCCCUG
462 GUAUCAG CUGAUGAGGCCGAAAGGCCGAA AACCCCU
463 GGUAUCA CUGAUGAGGCCGAAAGGCCGAA AAACCCC
468 CAGAUGG CUGAUGAGGCCGAAAGGCCGAA AUCAGAA
473 GCUCGCA CUGAUGAGGCCGAAAGGCCGAA AUGGUU
491 AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACUGGGC
496 GGAGAAG CUGAUGAGGCCGAAAGGCCGAA AGCCGAC
497 UGGAGAA CUGAUGAGGCCGAAAGGCCGAA AAGCCGA
499 AUUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAAGCC
500 CAUUGGA CUGAUGAGGCCGAAAGGCCGAA AAGAAGC
502 CACAUUG CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
511 AGCAGAU CUGAUGAGGCCGAAAGGCCGAA ACACAUU
514 GAAAGCA CUGAUGAGGCCGAAAGGCCGAA AUGACAC
519 UUUUCGA CUGAUGAGGCCGAAAGGCCGAA AGCAGAU
520 UUUUUCG CUGAUGAGGCCGAAAGGCCGAA AAGCAGA
521 AUUUUUC CUGAUGAGGCCGAAAGGCCGAA AAAGCAG
531 CAAGGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUU
537 CUUGUCC CUGAUGAGGCCGAAAGGCCGAA AGGGUGA
566 GUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCAGGU
599 CACAGAC CUGAUGAGGCCGAAAGGCCGAA ACAUCAG
602 GACCACA CUGAUGAGGCCGAAAGGCCGAA ACAACAU
609 UCCUGGG CUGAUGAGGCCGAAAGGCCGAA ACCACAG
618 CUCAGCC CUGAUGAGGCCGAAAGGCCGAA AUCCUGG
641 UGAUGGG CUGAUGAGGCCGAAAGGCCGAA AUCACCA
647 CGAAGAU CUGAUGAGGCCGAAAGGCCGAA AUGGGGA
650 UCCCGAA CUGAUGAGGCCGAAAGGCCGAA AUGAUGG
652 GAUCCCG CUGAUGAGGCCGAAAGGCCGAA AGAUGAU
653 GGAUCCC CUGAUGAGGCCGAAAGGCCGAA AAGAUGA
659 CAAACAG CUGAUGAGGCCGAAAGGCCGAA AUCCCGA
664 GAUGGCA CUGAUGAGGCCGAAAGGCCGAA ACAGGAU
665 GGAUGGC CUGAUGAGGCCGAAAGGCCGAA AACAGGA
671 CCAAGAG CUGAUGAGGCCGAAAGGCCGAA AUGGCAA
674 GCACCAA CUGAUGAGGCCGAAAGGCCGAA AGGAUGG
676 CAGCACC CUGAUGAGGCCGAAAGGCCGAA AGAGGAU
686 UGAUAAA CUGAUGAGGCCGAAAGGCCGAA ACCAGCA
688 UUUGAU CUGAUGAGGCCGAAAGGCCGAA AGACCAG
689 UUUUGAU CUGAUGAGGCCGAAAGGCCGAA AAGACCA
690 UUUUUGA CUGAUGAGGCCGAAAGGCCGAA AAAGACC
692 CCUUUUU CUGAUGAGGCCGAAAGGCCGAA AUAAAGA
720 GGGGCCU CUGAUGAGGCCGAAAGGCCGAA AUUGGUU
755 GAAAAUU CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
759 UCGGGAA CUGAUGAGGCCGAAAGGCCGAA AUUGAUC
760 GUCGGGA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU
761 CGUCGGG CUGAUGAGGCCGAAAGGCCGAA AAAUUGA
762 UCGUCGG CUGAUGAGGCCGAAAGGCCGAA AAAAUUG
771 CCAGGAA CUGAUGAGGCCGAAAGGCCGAA AUCGUCC

773	AGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAU CGU
774	GAGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAU CG
781	AGUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGG
795	UGCACUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
810	CCAUGUA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCC
811	UCCAUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUC
812	AUCCAUG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCU
830	CCUGGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCGGUU
855	GAGAUGC	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCU
860	GCACUGA	CUGAUGAGGCCGAAAGGCCGAA	AUGCGAC
862	CUGCACU	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCG
927	UCUGGCC	CUGAUGAGGCCGAAAGGCCGAA	ACUGCCU
981	CUGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUC CC
990	UGGCACU	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGC

Table BXII: Mouse CD40 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
18	GGUgucU u UGCCUCg	479	cAUCAcU U UUCgaaA
18	GGuguCU u UGCCucG	480	AUCacuU U UCGAAAA
24	UuUGCCU C gGCuGUG	481	UCacuUU U CGAAAAg
38	GCGcgCU a UGGGGCU	481	UCACuuU U cGaaAAG
62	CagcGGU c CaUCUag	492	AAAgUGU u AuCCcUG
62	CaGCgGU C CAUCuAG	560	CUaAUGU c aUCUGUG
66	gGUCCA U C uAGggCa	563	AUGUcaU C UGUGGUu
80	AGUGuGU u acgUGca	572	gUGGUuU a AagUCcC
80	AgUGUGU u AcgUGCa	572	GuGGUUU a aagUcCC
81	gUGugUU a CgUGCaG	577	UuAAagU c CCgGAUG
100	AAACAGU A CCUccac	620	UGGgcAU C CuCAUCA
126	CUGUgaU U UGUGCCA	626	UCCuCAU C AcCaUuu
127	UGUgaUU U GUGCCAG	632	uCAcCAU u UUCGGGg
170	CAGcUcU u gaGAaGA	632	UcaCCA U uUCggGG
208	gGCGAAU U CUCAGcC	634	AcCAUuU U CGGGgUg
209	GCGAAUU C ucAGcCc	635	CCaUuuU c GgGGUGu
233	gGGAGAU u cgcUgUC	635	cCAUuUU C GGGgUgu
267	ACCCAAU c AAggGcu	635	CCAuuuU C ggGGUGu
267	AcCCAAU c AaGggCu	647	UGuUucU C UaUAUCA
275	aAGGGCU U CGGGUua	649	uUucUCU a UAUCAAA
275	AaGGGcU U CgGgUua	651	ucUCUaU A UCAAAAA
276	AGGGCUU C GGGUuaA	653	UCUaUAU C AAAAAGG
281	UUCGGGU u aAGaAGg	735	gGAaGAU u aUCCcGG
281	UUCGGGU u AAGaAGg	759	cGCUGCU C CAGUGCA
314	ACACugU C UGuACCU	794	AgCCuGU C ACaCAGG
354	caAgGaU u GCgaGGC	794	AGcCuGU c acaCAGg
386	cCugUaU c CCUGGCU	819	AGAGAGU C GCAUCUC
394	CCUgGCU u uGgaGuu	824	GUCGCAU C UCAGUGC
394	CCuGGCU U UGgaGUu	826	CGCAUCU C AGUGCAG
395	CuGGCUU U GgaGUuA	876	cCCUGGU C UgAaCcC
429	caCUGAU A CCgUCUG	913	GGCUGCU U GCUGACC
434	AUACCgU C UGucAuC	997	CUCAaCU u GCuuUuu
434	AUaCcGU c UGuCAUC	1003	uUGCUUU u uAAGgAU
441	CugUCaU C CcuGCcC	1003	uugCUUU u uAaGGAU
452	GCCCAGU C GGCUUCU	1023	gaAAgCU c GGGCaUC
452	GCCCAGU C gGcuuCu	1048	CAGuGaU a UCUaccA
457	GUCGGCU U CUUCUCC	1052	gAUauCU a CCaaGuG
458	UCGGCUU C UUCUCCA	1081	CCAGagU u GuCUugc
460	GGCUUCU U CUCCA AU	1084	gAGUuGU C uUGCUGC
461	GCUUCUU C UCCA AUc	1086	gUugUCU U GcUGCgG
463	UUCUUCU C CAAUcaG	1097	gCgGcGU U CACUGuA
472	AAuCAGU C AucaCUu	1098	CgGcGUU C ACUGuAA
472	AAUcagU c auCACuU	1118	cgUgGCU A CAGGaGU

1118	CgUGGCU	a	CaggAgU
1141	CgCaGCU	u	gUGCUCG
1164	aCCUGgU	U	GCCAUCa
1202	UGuaaUU	a	UUUaUaC
1220	gGcAuCU	c	AgAAACu
1220	GGCAuCU	C	AGAAACu
1228	aGAaACU	c	UAgcaGG
1253	AaCaGGU	a	GUGgAAu
1331	AGgAGcU	U	GCUgCcc
1362	uUuUGaU	C	CCugGGA
1373	gGGaCUU	c	AUgguAA
1373	GgGACUU	c	AugguaA
1413	uUGUCAU	u	UGaccUC
1443	GUaaUGU	a	CcccGUG
1470	CACAuAU	c	CUaaaAu
1492	GugGUGU	a	uUGuAga
1497	GuAuUGU	A	gaAaUuA
1508	auUauUU	a	aUCcGCC
1508	AUuAuUU	a	auCCGcC
1523	cuGGGuU	u	CUaccUG

Table BXIII: Mouse CD40 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
18	CGAGGCA CUGAUGAGGCCGAAAGGCCGAA AGACACC
18	CGAGGCA CUGAUGAGGCCGAAAGGCCGAA AGACACC
24	CACAGCC CUGAUGAGGCCGAAAGGCCGAA AGGCAAA
38	AGCCCCA CUGAUGAGGCCGAAAGGCCGAA AGCGCGC
62	CUAGAUG CUGAUGAGGCCGAAAGGCCGAA ACCGCTG
62	CUAGAUG CUGAUGAGGCCGAAAGGCCGAA ACCGCTG
66	UGCCCUA CUGAUGAGGCCGAAAGGCCGAA AUGGACC
80	UGCACGU CUGAUGAGGCCGAAAGGCCGAA ACACACU
80	UGCACGU CUGAUGAGGCCGAAAGGCCGAA ACACACU
81	CUGCACG CUGAUGAGGCCGAAAGGCCGAA AACACAC
100	GUGGAGG CUGAUGAGGCCGAAAGGCCGAA ACUGUUU
126	UGGCACA CUGAUGAGGCCGAAAGGCCGAA AUCACAG
127	CUGGCAC CUGAUGAGGCCGAAAGGCCGAA AAUCACA
170	UCUUCUC CUGAUGAGGCCGAAAGGCCGAA AGAGCUG
208	GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AUUCGCC
209	GGGUGA CUGAUGAGGCCGAAAGGCCGAA AAUUCGC
233	GACAGCG CUGAUGAGGCCGAAAGGCCGAA AUCUCCC
267	AGCCCTU CUGAUGAGGCCGAAAGGCCGAA AUUGGGU
267	AGCCCTU CUGAUGAGGCCGAAAGGCCGAA AUUGGGU
275	UAACCCG CUGAUGAGGCCGAAAGGCCGAA AGCCCTU
275	UAACCCG CUGAUGAGGCCGAAAGGCCGAA AGCCCTU
276	UUAACCC CUGAUGAGGCCGAAAGGCCGAA AAGCCCTU
281	CCUUCUU CUGAUGAGGCCGAAAGGCCGAA ACCCGAA
281	CCUUCUU CUGAUGAGGCCGAAAGGCCGAA ACCCGAA
314	AGGUACA CUGAUGAGGCCGAAAGGCCGAA ACAGUGU
354	GCCUCGC CUGAUGAGGCCGAAAGGCCGAA AUCCUUG
386	AGCCAGG CUGAUGAGGCCGAAAGGCCGAA AUACAGG
394	AACUCCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
394	AACUCCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
395	UAACUCC CUGAUGAGGCCGAAAGGCCGAA AAGCCAG
429	CAGACGG CUGAUGAGGCCGAAAGGCCGAA AUCAGUG
434	GAUGACA CUGAUGAGGCCGAAAGGCCGAA ACGGUAU
434	GAUGACA CUGAUGAGGCCGAAAGGCCGAA ACGGUAU
441	GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AUGACAG
452	AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACUGGGC
452	AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACUGGGC
457	GGAGAAG CUGAUGAGGCCGAAAGGCCGAA AGCCGAC
458	UGGAGAA CUGAUGAGGCCGAAAGGCCGAA AAGCCGA
460	AUUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAAGCC
461	GAUUGGA CUGAUGAGGCCGAAAGGCCGAA AAGAAGC
463	CUGAUUG CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
472	AAGUGAU CUGAUGAGGCCGAAAGGCCGAA ACUGAUU
472	AAGUGAU CUGAUGAGGCCGAAAGGCCGAA ACUGAUU

479	UUUCGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUGAUG
480	UUUUCGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUGAU
481	CUUUUCG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUGA
481	CUUUUCG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUGA
492	CAGGGAU	CUGAUGAGGCCGAAAGGCCGAA	ACACUUU
560	CACAGAU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUAG
563	AACCACA	CUGAUGAGGCCGAAAGGCCGAA	AUGACAU
572	GGGACTU	CUGAUGAGGCCGAAAGGCCGAA	AAACCAC
572	GGGACTU	CUGAUGAGGCCGAAAGGCCGAA	AAACCAC
577	CAUCCGG	CUGAUGAGGCCGAAAGGCCGAA	ACTUUAA
620	UGAUGAG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCCA
626	AAUUGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGAGGA
632	CCCCGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGA
632	CCCCGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGA
634	CACCCCG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGU
635	ACACCCC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUGG
635	ACACCCC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUGG
635	ACACCCC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUGG
647	UGAUUA	CUGAUGAGGCCGAAAGGCCGAA	AGAAACA
649	UUUGAUA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAA
651	UUUUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGA
653	CCUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUAGA
735	CCGGGAU	CUGAUGAGGCCGAAAGGCCGAA	AUCUCC
759	UGCACUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCG
794	CCUGUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCU
794	CCUGUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCU
819	GAGAUGC	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCU
824	GCACUGA	CUGAUGAGGCCGAAAGGCCGAA	AUGCGAC
826	CUGCACU	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCG
876	GGGUUCA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
913	GGUCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCC
997	AAAAAGC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGAG
1003	AUCCUUA	CUGAUGAGGCCGAAAGGCCGAA	AAAGCAA
1003	AUCCUUA	CUGAUGAGGCCGAAAGGCCGAA	AAAGCAA
1023	GAUGCCC	CUGAUGAGGCCGAAAGGCCGAA	AGCUUUC
1048	UGGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AUCACUG
1052	CACUUGG	CUGAUGAGGCCGAAAGGCCGAA	AGAUUUC
1081	GCAAGAC	CUGAUGAGGCCGAAAGGCCGAA	ACUCUGG
1084	GCAGCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAACUC
1086	CCGCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGACAAC
1097	UACAGUG	CUGAUGAGGCCGAAAGGCCGAA	ACGCCGC
1098	UUACAGU	CUGAUGAGGCCGAAAGGCCGAA	AACGCCG
1118	ACUCCUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCACG
1118	ACUCCUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCACG
1141	CGAGCAC	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCG
1164	UGAUGGC	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGU
1202	GUUAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUA
1220	AGUUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCC
1220	AGUUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCC

1228	CCUGCUA	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCU
1253	AUUCCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCUGUU
1331	GGGCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCU
1362	UCCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUCAAAA
1373	UUACCAU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCC
1373	UUACCAU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCC
1413	GAGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGACAA
1443	CACGGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUAC
1470	AUUUUAG	CUGAUGAGGCCGAAAGGCCGAA	AUAUGUG
1492	UCUACAA	CUGAUGAGGCCGAAAGGCCGAA	ACACCAC
1497	UAAUUUC	CUGAUGAGGCCGAAAGGCCGAA	ACAAUAC
1508	GGCGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1508	GGCGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1523	CAGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AACCCAG

Table BXIV: Human B7 Hairpin Ribozyme and Target Sequence

nt. Position	Hairpin Ribozyme Sequence	Substrate
286	ACAGGCAG AGAA GAUGAC ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	GUCAUCA GCC CUGCCUGU
291	GCAAAACA AGAA GGGCUG ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	CAGCCCU GCC UGUUUUGC
295	AGGUGCAA AGAA GGCAGG ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	CTUGCCU GUU UUGCACCUCU
437	GCACCAAG AGAA GAAAGA ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	UCUUUCA GCU CUUGGUGC
469	AACACCTUG AGAA GAAGUG ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	CACUUCU GUU CAGGUGUU
518	GACCACAG AGAA GCGUUG ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	CAACGCU GUC CUGUGGUC
540	AGCUCUUC AGAA GAAACA ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	UGUUUCU GUU GAAAGAGCU
596	ACAUCAUA AGAA GCACCA ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	UGGUGCU GAC UAUGAUGU
644	CAAAGAUG AGAA GGUUCU ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	AGAACCG GAC CAUCUUUG
702	GUGCCCTUC AGAA GAUGGG ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	CCCAUCU GAC GAGGGCAC
795	GUAGGGAA AGAA GCUUUG ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	CAAAGCU GAC UUCCTUAC
819	AUUUCAAA AGAA GAUUAU ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	UAUAUCU GAC UUUGAAAU
939	UCTUUGGA AGAA GUUGUG ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	CACAACA GUU UCCCAAGA
1012	ACACAUGA AGAA GUGGUU ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	AACCACA GCU UCAUGUGU
1055	AGUUGAAG AGAA GAUUCA ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	UGAAUCA GAC CUUCAACU
1103	AGGAUGGG AGAA GGUUAU ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	AUAACCU GCU CCCAUCCU
1159	GUAGGUCA AGAA GCAUUA ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	AUAUGCU GCC UGACCTUAC
1163	AGCAGUAG AGAA GGCAGC ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	GCUGCCU GAC CUACUGCU
1171	UGGGGCAA AGAA GUAGGU ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	ACCUACU GCU UUGCCCCA
1356	GUGGGUAA AGAA GCUUAA ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	UUAAAGCU GUU UUACCCAC
1395	UCAGCUUA AGAA GAAAGA ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	UCUUUCA GAU UAAGCTUGA

Table BXV: Mouse B7 Hairpin Ribozyme and Target Sequence

nt. Position	Hairpin Ribozyme Sequence		Substrate
74	AGAAUUG	AGAA GAGUGU ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	ACACUCU GUU CCAUUCU
114	AUCCACCC	AGAA GAUGCU ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	AGCAUCU GCC GGGUGGAU
154	AAUCGAGA	AGAA GAGAUG ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	CAUCUCU GUU UCUCGAUU
265	CCUGCAUC	AGAA GACAUA ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	AUUGUCA GUU GAUGCAGG
328	GACGAUUC	AGAA GCACAA ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	UUGUGCU GCU GAUUCGUC
331	AAAGACGA	AGAA GCAGCA ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	UGCUGCU GAU UCGUCUUU
356	UCAUCAAC	AGAA GAAGAC ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	GUCUUCA GAU GUGAUGA
373	CUGACUUG	AGAA GUUGUU ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	AACAACU GUC CAAGUCAG
403	AACGGCAA	AGAA GCAUUA ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	UAUUGCU GCC UUGCCGUU
481	CAUUGACA	AGAA GCACCA ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	UGGUGCU GUC UGUCAUUG
529	CAUAUAAA	AGAA GGUUCU ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	AGAACC GAC UUAUAUUG
584	GUGCCCCG	AGAA GAAAGG ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	CCUUUCA GAC CCGGGCAC
600	AACGACAC	AGAA GUUUGU ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	ACAUACA GCU GUGUCGUU
677	GUAGAGAA	AGAA GCUUUG ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	CAAAGCU GAC UUCUCUAC
741	GGAAGCAA	AGAA GGUAUU ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	AUUACCU GCU UUGCUUCC
1028	AUGACGAC	AGAA GUUAUU ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	AUAACA GUC GUGGUCAU
1077	UCUUCUGA	AGAA GCUUCU ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	AGAAGCU GUU UCAGAAGA
1116	GAAGGUAA	AGAA GUUGUU ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	AACAACA GCC UUAACUUC
1153	GGAAGACG	AGAA GUUCAG ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	CUGAACA GAC CGUCUUC
1157	UAAAGGAA	AGAA GUCUGU ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	ACAGACC GUC UUCUUUA
1178	CCCACAUG	AGAA GAGAAG ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	CUUCUCU GUC CAUGUGGG
1246	UCCGAAAG	AGAA GCUAGC ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	GCUAGCU GAU CUUUCGGA
1523	CAGAAAAG	AGAA GGCCUC ACCAGAGAAACACACGUGUGGUAUUAUACUUGGUA	GAGGCTU GCC CUUUUCUG

Table BXVI: Human B7-2 Hairpin Ribozyme and Target Sequences

nt. Position	HP Ribozyme Sequences		Substrate
25	GUUACAGC	AGAA GAGAAG ACCAGAGAAACACACACGUGUGUGGUA	CUUCUCU GCU GCUGUAC
28	CCUGUUAC	AGAA GCAGAG ACCAGAGAAACACACACGUGUGGUA	CUCUGCU GCU GUUACAGG
57	CCCCACUC	AGAA GUGUGU ACCAGAGAAACACACACGUGUGGUA	ACACACG GAU GAGUGGG
162	CACCAGAG	AGAA GGAAGG ACCAGAGAAACACACACGUGUGGUA	CCUUCUU GCU CUCUGGUG
175	UUCAGAGG	AGAA GCACCA ACCAGAGAAACACACACGUGUGGUA	UGGUGCU GCU CCUCUGAA
214	CAUGGCAG	AGAA GCAGUC ACCAGAGAAACACACACGUGUGGUA	GACUGCA GAC CUGCCAU
380	CAGGGUCC	AGAA GUCCGA ACCAGAGAAACACACACGUGUGGUA	UCCGACA GUU GGACCCUG
408	UGUCCUUG	AGAA GAAGAU ACCAGAGAAACACACACGUGUGGUA	AUCUUA GAU CAAGGACA
480	CAGAAUUC	AGAA GGUGGA ACCAGAGAAACACACACGUGUGGUA	UCCACCA GAU GAUUCUG
575	UAUAGAUG	AGAA GGUCAA ACCAGAGAAACACACACGUGUGGUA	UUGACCU GCU CAUCUAUA
710	AACAGACA	AGAA GAUGGA ACCAGAGAAACACACACGUGUGGUA	UCCAUA GCU UGUCUGUU
718	GGGAUGA	AGAA GACAAG ACCAGAGAAACACACACGUGUGGUA	CUUGUCU GUU UCAUUCUU
730	CUUGUAC	AGAA GGGAAU ACCAGAGAAACACACACGUGUGGUA	AUUCUUU GAU GUUACGAG
783	AAGAUAUA	AGAA GCGUCU ACCAGAGAAACACACACGUGUGGUA	AGACGGG GCU UUUUUCUU
825	CUGGGGGA	AGAA GAGGGU ACCAGAGAAACACACACGUGUGGUA	ACCTUCA GCC UCCCCCAG
835	GGAAUGUG	AGAA GGGGGA ACCAGAGAAACACACACGUGUGGUA	UCCCCCA GAC CACAUUCC
856	GGAAUAC	AGAA GUAAUC ACCAGAGAAACACACACGUGUGGUA	GAUUA GAU GUACUUCU
896	UAGAAUUA	AGAA GAAAC ACCAGAGAAACACACACGUGUGGUA	GUUUUCU GUC UAAUUCUA
930	AGUUGCGA	AGAA GCUUCU ACCAGAGAAACACACACGUGUGGUA	AGAAGG GCC UCGCAACU
987	UUUUCUUG	AGAA GUUCAC ACCAGAGAAACACACACGUGUGGUA	GUGAACA GAC CAAGAAAA
1027	UGGGCUUC	AGAA GAUUCU ACCAGAGAAACACACACGUGUGGUA	AAGAUCU GAU GAAGCCCA

Table BXVII: Mouse B7-2 Hairpin Ribozyme and Target Sequences

nt. Position	HP Ribozyme Sequences	Substrate
10	UCUUAAGC AGAA GCUUGC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GCAAGCA GAC GCGUAAGA
42	UUGUUCAA AGAA GUGCUG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CAGCAGG GAC UUGAACAA
56	CUACAGGA AGAA GGUUGU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	ACAACCA GAC UCCUGUAG
108	CAUGGUGC AGAA GGGGUC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GACCCCA GAU GCACCAUG
146	AUCAGCAA AGAA GUCAAC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UGUGACA GUC UUGCUGAU
154	CAUCUGAG AGAA GCAAGA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UCUUGCU GAU CUCAGAUG
161	GAACAGC AGAA GAGAUC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GAUTUCA GAU GCUUUUUC
167	UCCACGGA AGAA GCAUCU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AGAUGCU GUU UCCGUGGA
211	AUGGGCAC AGAA GAUAUG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CAUAUCU GCC GUGCCCAU
400	UGUCCUUG AGAA GAACAU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AUGTUCA GAU CAAGGACA
679	AGAUACTUG AGAA GUUCUG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CAGAACTU GUU CAGUAUCTU
696	AAGAGAGA AGAA GUUGGA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UCCAACA GCC UCUCUCU
716	CACACACC AGAA GGGAAU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AUUCGCG GAU GUGUGUGUG
737	ACACACAC AGAA GUCAUA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UAUGACC GUU GUGUGUGU
839	GUAACTUGA AGAA GUAAUC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GAUUACA GCU UCAGUUAC
874	CAUUGAUG AGAA GCAUCA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UGAUGCU GCU CAUCAUTUG
907	GCCUGCUA AGAA GAUUCG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CGAAUCA GCC UAGCAGGC
929	AACUUAGA AGAA GUGUUG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CAACACA GCC UCUAAGUU
1115	UUCCAAUC AGAA GAGAAC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GUUTUCA GCU GAUUGGAA
1118	GAUUCUCA AGAA GCUAG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CUCAGCU GAU UGGAUUC
1133	AAUUAUUC AGAA GUAGAA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UUUUAUA GUU GAAUUAU

Table BXVIII : Human CD40 Hairpin Ribozyme and Target Sequences

nt. Position	Hairpin Ribozyme Sequences	Substrate
26	GACCAGGC AGAA GGACCA ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	UGGUCCU GCC GCUUGGUC
29	UGAGACCA AGAA GCAGGA ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	UCCUGCC GCC UGGUCUCA
58	ACUGCAGA AGAA GACGAA ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	UUCGUUCU GCC UCUGCAGU
84	GGUCAGCA AGAA GCCCCA ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	UGGGGCU GCU UGCTUGACC
91	GGACAGCG AGAA GCAAGC ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	GCUUGCU GAC CGCTUGUCC
95	GGAUUGAC AGAA GUCAGC ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	GCTUGACC GCU GUCCAUC
98	UCUGGAUG AGAA GCGGUC ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	GACCGCU GUC CAUCCAGA
159	GCACAAAG AGAA GCACUG ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	CAGUGCU GUU CUUUGUGC
414	CGAGCAUG AGAA GUGCAG ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	CUGCAOC GCU CAUGCTUG
429	GACCCCA AGAA GGGCGA ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	UCGCCCG GCU UUGGGGUC
445	CUGUAGCA AGAA GCUUGA ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	UCAAGCA GAU UGCTUACAG
483	GCCGACUG AGAA GGGTUC ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	GAGCCCU GCC CAGUCGGC
488	AAGAAGCC AGAA GGGCAG ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	CUGCCCA GUC GGCUUCU
492	GGAGAAGA AGAA GACUGG ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	CCAGUOG GCU UCUUCUCC
515	UUUUCGAA AGAA GAUGAC ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	GUCAUCU GCU UUCGAAAA
593	CAGACAAC AGAA GUCUUG ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	CAAGACU GAU GUUGUCUG
619	GGGCTUC AGAA GAUCCU ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	AGGAUCG GCU GAGAGCCC
661	GGAUUGCA AGAA GGAUCC ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	GGAUCCU GUU UGCCAUCC
764	GGNAGAUC AGAA GGAATA ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	UUUUCU GAC GAUCUUC
788	ACUGGAGC AGAA GUGUUG ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	CAACACU GCU GCUCCAGU
791	UGCACUGG AGAA GCAGUG ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	CACUGCU GCU CCAGUGCA
924	CUUGGCC AGAA GCTUGU ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	ACAGGCA GUU GGCCAGAG
946	CTUGCAGC AGAA GCACCA ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	UGGUGCU GCU GCTUGCAGG
949	ACCCUUGC AGAA GCAGCA ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA	UGCUGCU GCU GCAGGGGU

Table BXIX: Mouse CD40 Hairpin Ribozyme and Substrate Sequences

nt. Position	HP Ribozyme Sequences	Substrate
25	GGCGGCAC AGAA GAGGCA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGCCUUG GCU GUGCGCGC
45	UGUCAACA AGAA GCGCCA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGGGGCU GCU UGUUGACA
59	CCUAGAUG AGAA GCUGUC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GACAGCG GUC CAUCUAGG
144	GCUGUCA AGAA GCUUCC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GGAGCC GAC UGACAAAGC
164	UUCUCAAG AGAA GUGCAG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CUGCACA GCU CUUGAGAA
212	UUCCACUG AGAA GAGAAU ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AUUCUCA GCC CAGUGGAA
311	CAGGUACA AGAA GUGUCU ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AGACACU GUC UGUACCTUG
431	GGAUGACA AGAA GUAUCA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGAUACC GUC UGUCAUCC
444	GCCGACUG AGAA GGGNUG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CAUCCCU GCC CAGUCGCG
449	AAGAAGCC AGAA GGGCAG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CUGCCCA GUC GGCUCUCU
453	GGAGAAGA AGAA GATUGG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CCAGUOG GCU UCUUCUCC
550	UGACAUUA AGAA GATUCG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CGAGUCA GAC UAAUGUCA
580	GGGCUCCG AGAA GGGACU ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AGUCCCG GAU GCGAGCCC
592	GAUAGACC AGAA GGGCUC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GAGCCCU GCU GGUCAUUC
605	CCCAUCAC AGAA GGAUUG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CAUUCU GUC GUGAUGGG
701	UGCCGUCG AGAA GCAGGG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CCUUGOG GCU CGACGGCA
752	ACUGGAGC AGAA GUGUUA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UAAACCC GCU GCUCCAGU
755	UGCACUGG AGAA GCGGUG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CACCCGU GCU CCAGUGCA
787	GUGUGACA AGAA GACACC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GGUGUCA GCC UGUACACAC
890	CUUCCAAA AGAA GUUCCA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGGAACTU GCU UUUUGGAGG
909	GGUCAGCA AGAA GCCAUC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GAUGGCU GCU UGCUGACC
916	UUCAAAAG AGAA GCAAGC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GCUUGCU GAC CUUUUGAA
975	UGACAGGG AGAA GGCAUG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CAUGCCU GCC CCCUGUCA
1137	CGAGCACA AGAA GCGGGC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GCCCCGA GCU UGUGCUOG
1276	GUUUUAAA AGAA GUUUUC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AGAAACA GCU UUUAAAAC
1334	CGGGUUUG AGAA GCAAGC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GCTUGCU GCC CAAACCCG
1352	GGAUCAAA AGAA GGUAAAC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GUUACCU GAU UUUUGAUCC
1512	AAACCCAG AGAA GAUUA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UUAUCC GCC CUGGGUUU

Table CII: 2.5 μ mol RNA Synthesis Cycle

Reagent	Equivalents	Amount	Wait Time*
Phosphoramidites	6.5	163 μ L	2.5
S-Ethyl Tetrazole	23.8	238 μ L	2.5
Acetic Anhydride	100	233 μ L	5 sec
N-Methyl Imidazole	186	233 μ L	5 sec
TCA	83.2	1.73 mL	21 sec
Iodine	8.0	1.18 mL	45 sec
Acetonitrile	NA	6.67 mL	NA

* Wait time does not include contact time during delivery.

Table EVII: Deprotection of a 36 mer all ribo oligo using 70% ethylamine in aqueous. The data are as follows upon HPLC reprocessing:

Sample	OD's	% Full Length Product (FLP)	% frontside	%backside
MA 10'@65°	0.984	14.5073	71.6740	13.8186
MA 10'@65°	1.125	18.9269	67.8006	13.2725
EA rt 10'	0.925	16.5804	66.8186	16.6010
EA rt 10'	0.920	15.7421	67.5794	16.6785
EA rt 30'	0.971	17.4694	67.6782	14.8525
EA rt 30'	0.794	15.7587	69.8084	14.4329
EA 40° 10'	0.819	18.0827	66.4937	15.4236
EA 40° 10'	0.986	17.5763	66.7865	15.6372
EA 40° 15'	0.877	18.7963	67.0064	14.1999
EA 40° 15'	0.911	18.7808	70.7306	10.4885
EA 55° 10'	1.001	17.8810	66.4703	15.6487
EA 55° 10'	1.023	19.1069	68.6706	12.2225

Claims

1. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein
5 said nucleic acid comprises a 2'-C-allyl modification at position No. 4 of said nucleic acid, and wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'- end modification.
2. The enzymatic nucleic acid of claim 1, wherein said nucleic acid
10 comprises a 3'-3' linked inverted ribose moiety at said 3' end.
3. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises a 2'-amino modification at position No. 4 and/or at position No. 7 of said nucleic acid, wherein said nucleic acid
15 comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.
4. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein
20 said nucleic acid comprises a non-nucleotide substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.
- 25 5. An enzymatic nucleic acid which cleaves target mRNA having a sequence selected from SEQ. ID. NOS. 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, and 281, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises a 6-methyl
30 uridine substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid comprises at least

ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.

- 5 6. The enzymatic nucleic acid which cleaves target mRNA having a sequence selected from SEQ. ID. NOS. 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, and 281, wherein said nucleic acid comprises of at least five ribose residues, wherein said nucleic acid comprises a 2'-C-allyl modification at position No. 4 of the said nucleic acid, wherein said
- 10 nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 2'-3' linked inverted ribose or thymidine moiety at its 3' end.
- 15 7. The enzymatic nucleic acid of any one of claims 1-6, wherein said nucleic acid comprises phosphorothioate linkages at least three of the seven 5' terminal nucleotides.
8. Nucleic acid molecule which blocks synthesis and/or expression of an mRNA encoding B7-1, B7-2, B7-3 and/or CD40.
9. The nucleic acid of claim 8, wherein said molecule is an enzymatic nucleic acid molecule.
- 20 10. The nucleic acid molecule of claim 9, wherein, the binding arms of said enzymatic nucleic acid contain sequences complementary to the nucleotide base sequences in any one of Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX.
- 25 11. The nucleic acid molecule of claims 9 or 10, wherein said nucleic acid molecule is in a hammerhead motif.
12. The enzymatic nucleic acid molecule of claim 9 or 10, wherein said nucleic acid molecule is in a hairpin, hepatitis Delta virus, group I intron, VS nucleic acid or RNaseP nucleic acid motif.

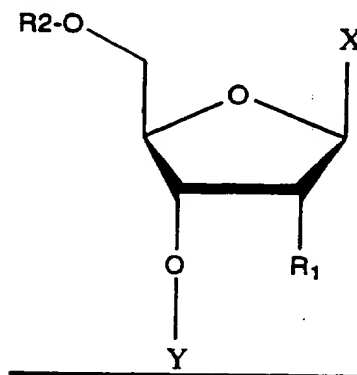
13. The enzymatic nucleic acid molecule of any of claims 9 or 10, wherein said ribozyme comprises between 12 and 100 bases complementary to the RNA of said region.
- 5 14. The enzymatic nucleic acid of claim 13, wherein said ribozyme comprises between 14 and 24 bases complementary to the RNA of said region.
- 15 15. Enzymatic nucleic acid molecule consisting essentially of any ribozyme sequence selected from those shown in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII.
- 10 16. A mammalian cell including an enzymatic nucleic acid molecule of any of claims 8 or 9.
17. The cell of claim 16, wherein said cell is a human cell.
- 15 18. An expression vector comprising nucleic acid encoding the enzymatic nucleic acid molecule of any of claims 9 or 10, in a manner which allows expression and/or delivery of that enzymatic RNA molecule within a mammalian cell.
19. A mammalian cell including an expression vector of claim 18.
- 20 20. The cell of claim 19, wherein said cell is a human cell.
21. A method for treatment of a patient having a condition associated with the level of B7-1, B7-2, B7-3 and/or CD40, wherein the patient, tissue donor or population of corresponding cells is administered a therapeutically effective amount of an enzymatic nucleic acid molecule of claims 8, 9 or 10.
- 25 22. A method for treatment of a condition related to the level of B7-1, B7-2, B7-3 and/or CD40 activity by administering to a patient an expression vector of claim 21.
23. The method of claims 21 or 22, wherein said patient is a human.

24. A method for inducing tolerance in a recipient to alloantigen of a donor comprising treating antigen presenting cells from a donor with nucleic acid of claim 8 or 9, and infusion of said treated antigen presenting cells into said recipient.
- 5 25. A method for enhancing graft tolerance comprising contacting a nucleic acid of claims 8 or 9 with cells of said graft prior to transplantation.
26. A method for treatment of an autoimmune disease, comprising contacting an antigen presenting cell of a patient with a nucleic acid of claims 8 or 9.
- 10 27. The method of claim 26, wherein said cells are contacted *ex vivo* with said nucleic acid.
28. The method of claim 26, wherein said cells are contacted with autoantigen characteristic of said disease.
- 15 29. The method of claim 28, wherein said cells are reinfused into said patient.
30. Enzymatic nucleic acid having at least one modified base substitution, wherein said base substitution is selected from a group comprising pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyluracil, dihydrouracil, naphthyl, 6-methyl-uracil and aminophenyl.
- 20 31. The enzymatic nucleic acid of any of claim 30, wherein said nucleic acid has a hammerhead motif.
32. Mammalian cell comprising an enzymatic nucleic acid molecule of and of claims 30-31.
- 25 33. The enzymatic nucleic acid of claim 31, wherein said nucleic acid includes said modified base substitutions at position 4 or at position 7.
34. The ribozyme of claim 33, wherein said substitution is 6-methyl uracil.
35. The ribozyme of claim 33, wherein said substitution is pyridin-4-one.

36. The ribozyme of claim 33, wherein said substitution is phenyl.
37. The ribozyme of claim 33, wherein said substitution is pyridin-2-one.
38. The ribozyme of claim 33, wherein said substitution is pseudouracil.
39. The ribozyme of claim 33, wherein said substitution is 2, 4, 6-trimethoxy
5 benzene.
40. The ribozyme of claim 33, wherein said substitution is dihydrouracil.
41. The ribozyme of claim 33, wherein said substitution is 3-methyluracil.
42. The ribozyme of claim 33, wherein said substitution is naphthyl.
43. The ribozyme of claim 33, wherein said substitution is aminophenyl.
- 10 44. 2'-deoxy-2'-alkylnucleoside.
45. 2'-deoxy-2'-alkylnucleotide.
46. Oligonucleotide comprising one or more 2'-deoxy-2'-alkylnucleotides.
47. Enzymatic nucleic acid comprising a 2'-deoxy-2'-alkylnucleotide.
- 15 48. Method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group.
49. 2'-deoxy-2'-alkylnucleotide triphosphate.
- 20 50. Method for synthesis of a 2'-C-allyl derivative from a 5'-O-DMT-3'-O-TBDMS-base comprising the steps of:
- (a) phenoxytriacylation of 5'-O-DMT-3'-O-TBDMS-base to yield a thioester, replacing a 2' hydroxyl group with a phenoxythiocarbonyl group, and

(b) Heck acylation of said thioester to form a 2'-C-allyl derivative in which said 2'-phenoxythiocarbonyl group is replaced with said 2'-C-alkyl group to yield said 2'-C-allyl derivative.

51. A compound having the formula:



5

wherein, R1 represents 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl; X represents a base or H; Y represents a phosphorus-containing group; and R2 represents O, DMT or a phosphorus-containing group.

52. Oligonucleotide comprising one or more compounds of claim 51.

10 53. Enzymatic nucleic acid comprising a compound of claim 51.

54. The compound of claim 51, wherein said compound is in the form of a triphosphate.

55. Enzymatic nucleic acid of claim 53 wherein said nucleic acid is in a hammerhead motif.

15 56. Enzymatic nucleic acid of claim 53, wherein said nucleic acid is in a hairpin, hepatitis delta virus, group I intron, VS RNA or RNase P RNA motif.

57 57. Enzymatic nucleic acid of claim 55, wherein said hammerhead ribozyme has positions 4 and/or 7 substituted with 2'-O-methylthiomethyl.

20

58. Enzymatic nucleic acid of claim 55 or 57, wherein one monomer in stem II of said hammerhead is substituted with at least one 2'-O-methylthiomethyl.
59. Enzymatic nucleic acid of claim 55 or 56, wherein said nucleic acid is substituted at one or more positions with 2'-O-methylthiophenyl.
60. A mammalian cell comprising a compound of any one of the claims 51-59.
61. The cell of claim 60, wherein said cell is a human cell.
62. Method for producing an enzymatic nucleic acid molecule having activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one position having at its 2'-position an 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl group.
64. Hammerhead ribozyme having a non-nucleotide in the catalytic core in a site selected from the group consisting of the normally occurring uracil at position 4 and 7.
65. Hammerhead ribozyme having a stem II and a loop II, wherein said loop II comprises a non-nucleotide.
66. Hammerhead ribozyme having a non-nucleotide at its 3' end.
67. A mammalian cell comprising an enzymatic nucleic acid molecule of any one of the claims 64-67.
68. The cell of claim 67, wherein said cell is a human cell.
69. Method of synthesis of abasic ribonucleoside mimetics described in figure 58.
70. A method for the deprotection of RNA comprising the step of providing aqueous ethylamine (EA) at between 25°C - 60°C for 5 to 30 minutes to remove any exocyclic amino protecting groups from protected RNA.

71. The method of claim 70 wherein, said ethylamine is provided at 40°C for 10 minutes.
72. The method of claim 70 wherein, said ethylamine is provided at 55°C for 10 minutes.
- 5 73. The method of claim 70, further comprising deprotection of RNA alkylsilyl protecting groups comprising, contacting said groups with anhydrous triethylamine•hydrogen fluoride (aHF•TEA) trimethylamine or diisopropylethylamine at between 60 °C-70 °C for 0.25-24 h.
- 10 74. The method of any one of claims 70-73 wherein, said RNA is an enzymatic RNA.
75. Method for synthesis of an enzymatic nucleic acid, comprising the steps of:
- 15 providing a 3' and a 5' portion of said enzymatic nucleic acid having independent chemically reactive groups at the 5' and 3' positions, respectively, under conditions in which a covalent bond is formed between said 3' and 5' portions by said chemically reactive groups, said bond being selected from the group consisting of, disulfide, morpholino, amide, ether, thioether, amine, a double bond, sulfonamide, ester, carbonate, hydrazone, said bond not being a
- 20 natural bond formed between a 5' phosphate group and a 3' hydroxyl group.
76. The method of claim 75, wherein said nucleic acid has a hammerhead motif and said 3' and 5' positions each have said chemically reactive groups in or immediately adjacent to the stem II region.
- 25 77. The method of claim 75, wherein one said chemically reactive group is $(CH_2)_nSH$ and the other chemically reactive group is $(CH_2)_nSH$, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 30 78. The method of claim 75, wherein one said chemically reactive group is $(CH_2)_nNH_2$ and the other chemically reactive group is ribose, wherein

each n independently is an integer from 0 to 10 inclusive and may be the same or different.

- 5 79. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$ and the other chemically reactive group is COOH , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 10 80. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{X}$ and the other chemically reactive group is $(\text{CH}_2)_n\text{OH}$ or $(\text{CH}_2)_n\text{SH}$; wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different; X is halogen.
81. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$ and the other chemically reactive group is CHO , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 15 82. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{PPh}_3$ and the other chemically reactive group is CHO , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 20 83. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$ and the other chemically reactive group is $(\text{CH}_2)_n\text{SO}_2\text{Cl}$, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 25 84. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{OH}$ and the other chemically reactive group is COOH , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 30 85. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{COH}$ and the other chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.

86. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{COX}$ and the other chemically reactive group is $(\text{CH}_2)_n\text{OH}$, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 5 87. The method of claim 78, wherein said conditions include provision of NaIO_4 in contact with said ribose, and subsequent provision of NaBH_4 or NaCNBH_3 .
88. The method of claim 79, wherein said conditions include provision of a coupling reagent.
- 10 89. A mixture comprising 5' and 3' portions of an enzymatic nucleic acid having a 3' and 5' chemically reactive group respectively selected from the group consisting of $(\text{CH}_2)_n\text{SH}$, $(\text{CH}_2)_n\text{NH}_2$, ribose, COOH , $(\text{CH}_2)_n\text{X}$, $(\text{CH}_2)_n\text{PPh}_3$, CHO , $(\text{CH}_2)_n\text{SO}_2\text{Cl}$, $(\text{CH}_2)_n\text{COX}$, $(\text{CH}_2)_n\text{X}$, $(\text{CH}_2)_n\text{OH}$, $(\text{CH}_2)_n\text{COH}$, and $(\text{CH}_2)_n\text{SH}$; wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different and X is halogen.
- 15 90. The method of claim 75, wherein one said chemically reactive group is linking group-SH and the other chemically reactive group is linking group-SH, wherein each linking group may be the same or different.
- 20 91. The method of claim 75, wherein one said chemically reactive group is linking group- NH_2 and the other chemically reactive group is ribose.
92. The method of claim 75, wherein one said chemically reactive group is linking group- NH_2 and the other chemically reactive group is COOH .
- 25 93. The method of claim 75, wherein one said chemically reactive group is linking group- X and the other chemically reactive group is linking group- OH or linking group- SH ; wherein each linking group may be the same or different; X is halogen.
94. The method of claim 75, wherein one said chemically reactive group is linking group- NH_2 and the other chemically reactive group is CHO .

95. The method of claim 75, wherein one said chemically reactive group is linking group-PPh₃ and the other chemically reactive group is CHO.
96. The method of claim 75, wherein one said chemically reactive group is linking group-NH₂ and the other chemically reactive group is linking group-SO₂Cl, wherein each linking group may be the same or different.
97. The method of claim 75, wherein one said chemically reactive group is linking group-OH and the other chemically reactive group is COOH.
98. The method of claim 75, wherein one said chemically reactive group is linking group-COH and the other chemically reactive group is linking group-NH₂, wherein each linking group may be the same or different.
99. The method of claim 75, wherein one said chemically reactive group is linking group-COX and the other chemically reactive group is linking group-OH, wherein each linking group may be the same or different.
100. The method of claim 91, wherein said conditions include provision of NaIO₄ in contact with said ribose, and subsequent provision of NaBH₄ or NaCNBH₃.
101. The method of claim 100, wherein said conditions include provision of a coupling reagent.
102. A mixture comprising 5' and 3' portions of an enzymatic nucleic acid having a 3' and 5' chemically reactive group respectively selected from the group consisting of linking group-SH, linking group-NH₂, ribose, COOH, linking group-X, linking group-PPh₃, CHO, linking group-SO₂Cl, linking group-COX, linking group-X, linking group-OH, linking group-COH, and linking group-SH; wherein each linking group may be the same or different and X is halogen.
103. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said

stem comprises at least 8 base pairs wherein said molecule is transcribed by a RNA polymerase II promoter system.

5 104. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is transcribed by a U6 small nuclear RNA promoter system.

10 105. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is transcribed by an adenovirus VA1 RNA promoter system.

15 106. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is a
20 chimeric adenovirus VA1 RNA.

25 107. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said intramolecular stem is separated from said desired RNA by a spacer sequence.

108. The RNA molecule of claim 107, wherein said spacer sequence is about 5-50 nucleotides.

FIG. 1.

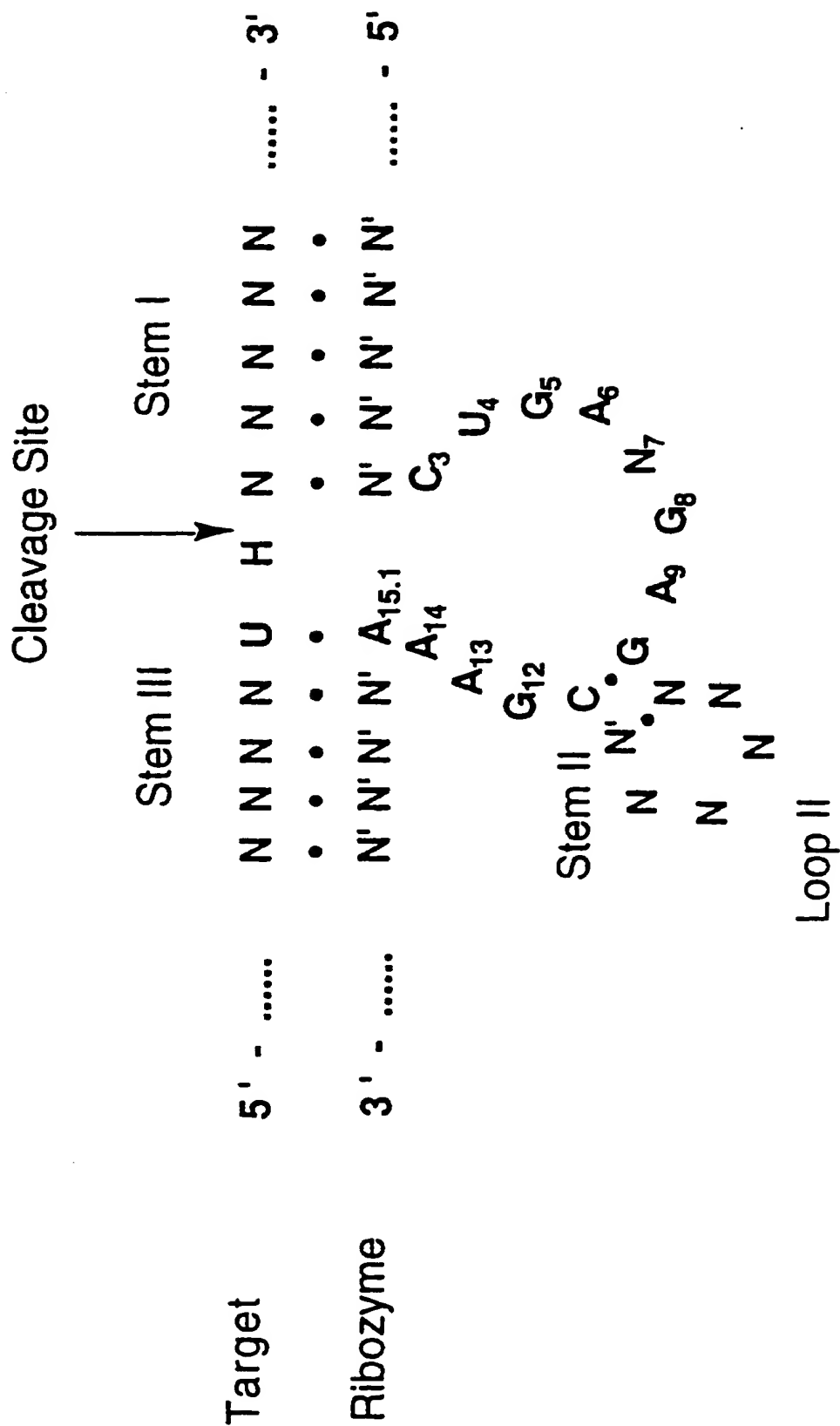


FIG. 2a.

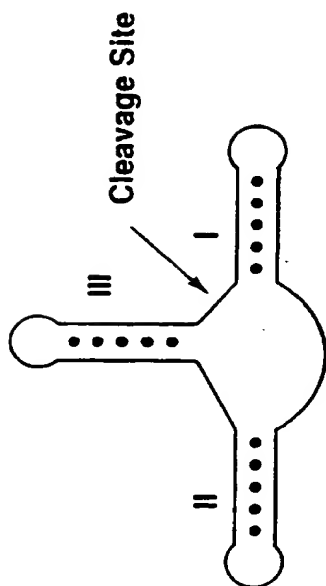


FIG. 2b.

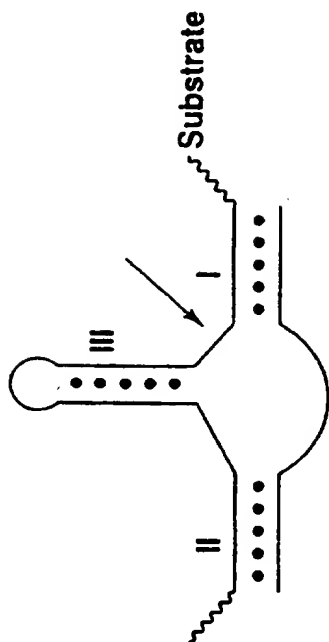


FIG. 2c.

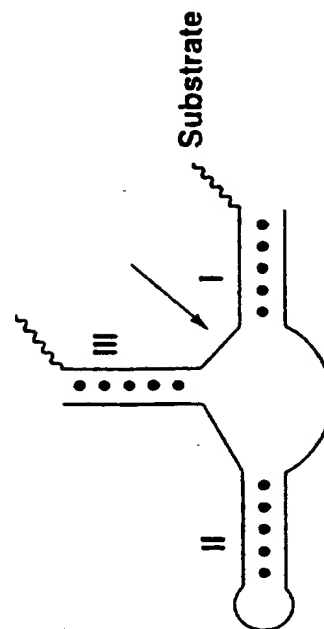
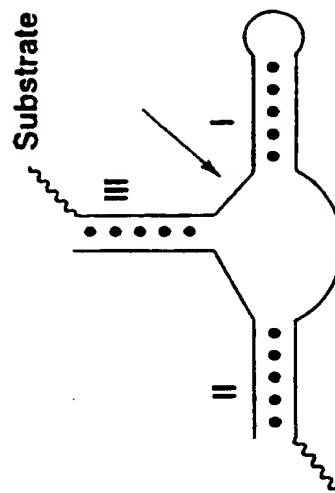


FIG. 2d.



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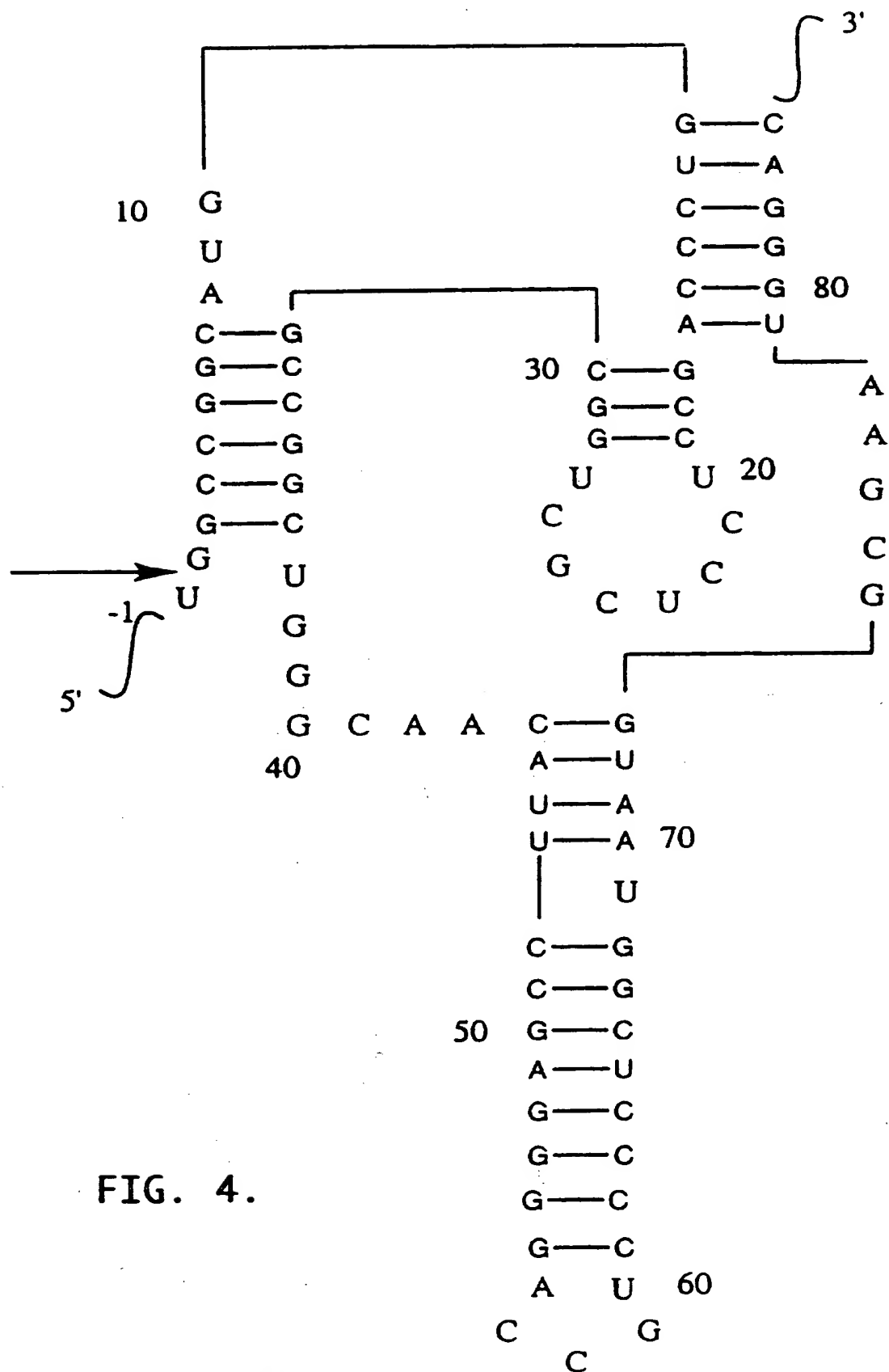
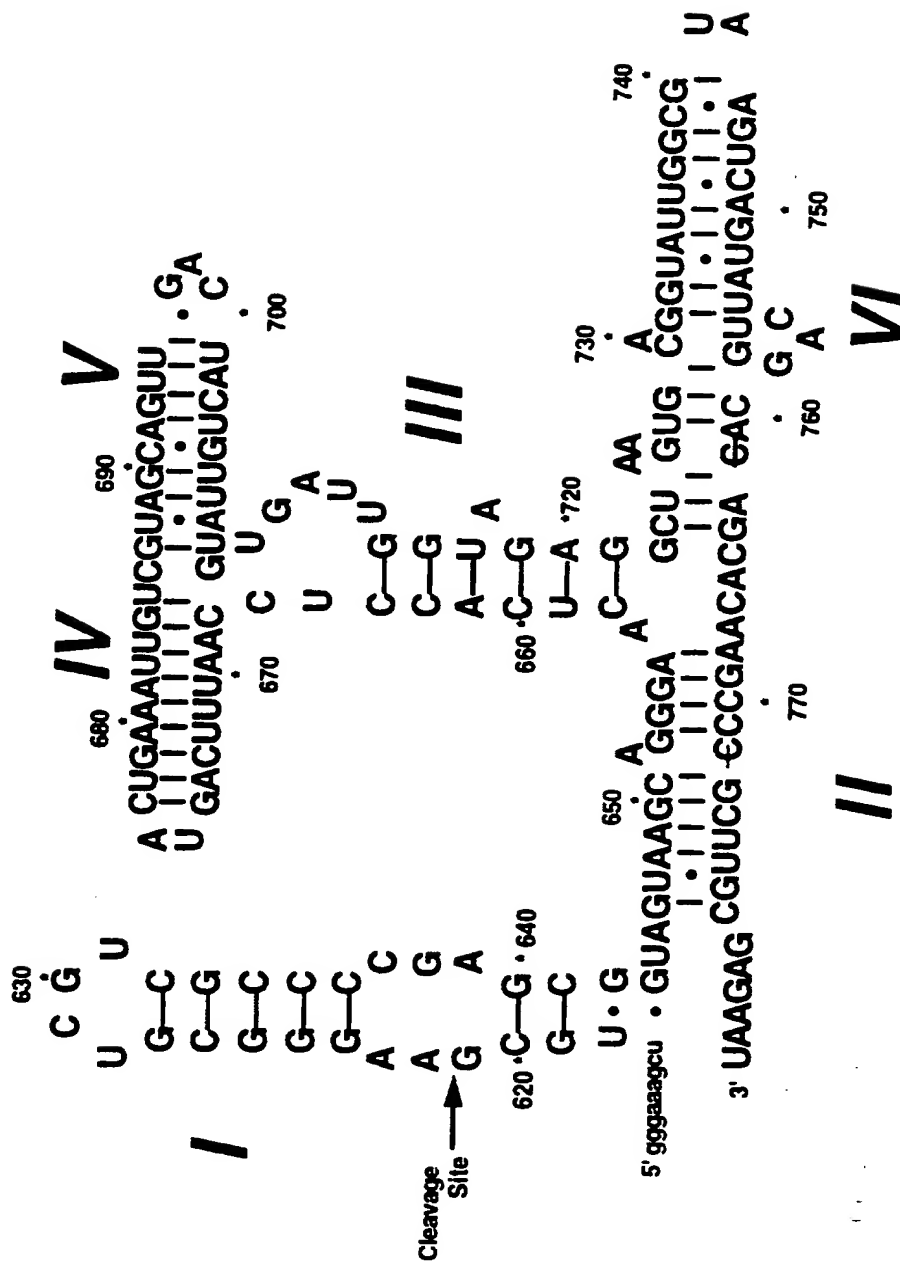


FIG. 4.

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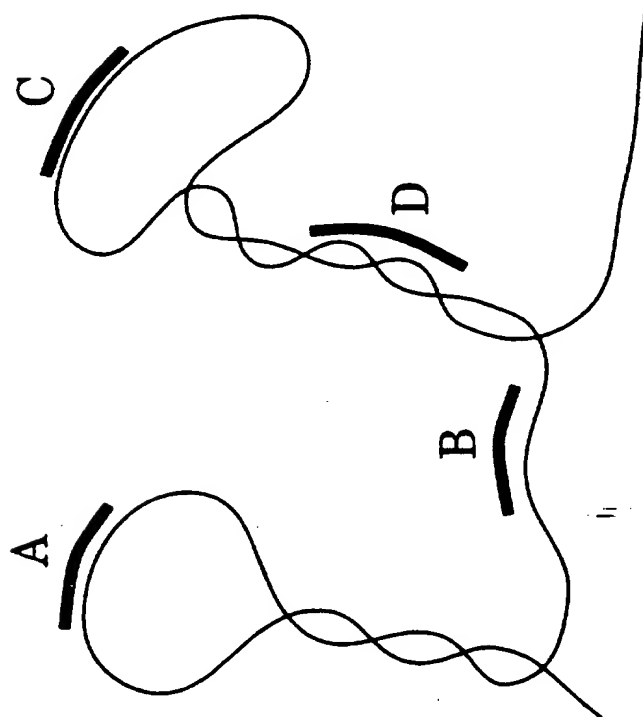
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FIG. 5.



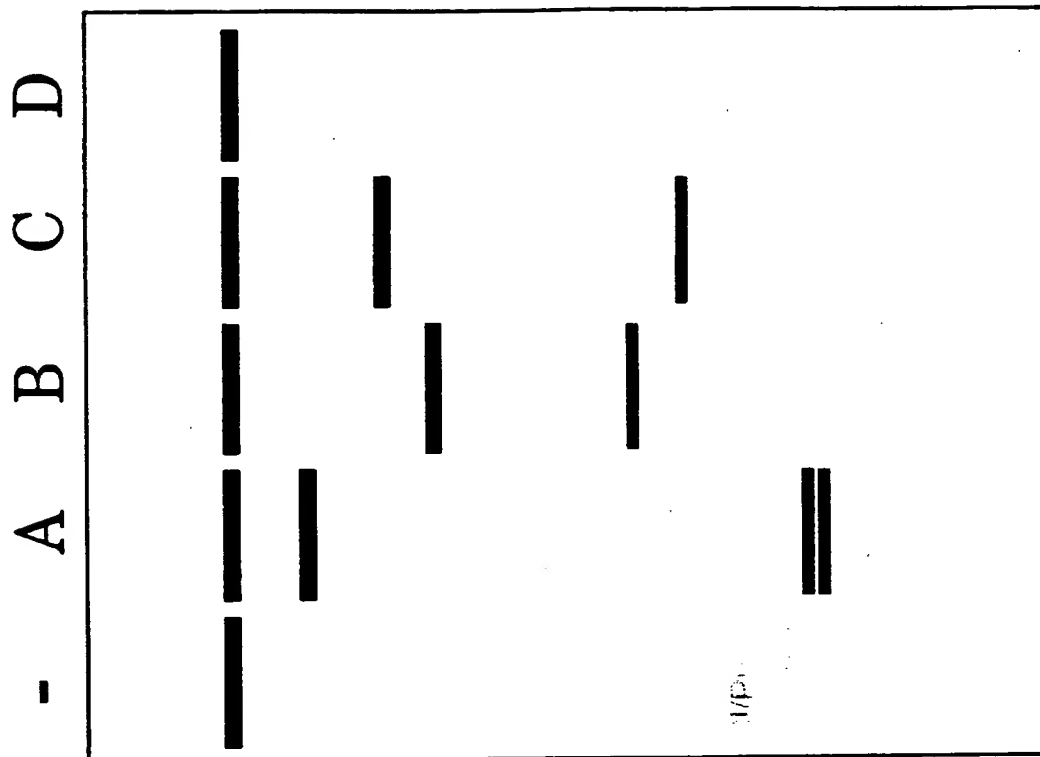
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FIG. 6a.



- Body-labeled transcript
(not purified)
- DNA oligo
(10 nM, 100 nM and 1000 nM)
- RNase H
(0.08 - 1.0 u/μl)
- 37°C, 10 min

FIG. 6b.



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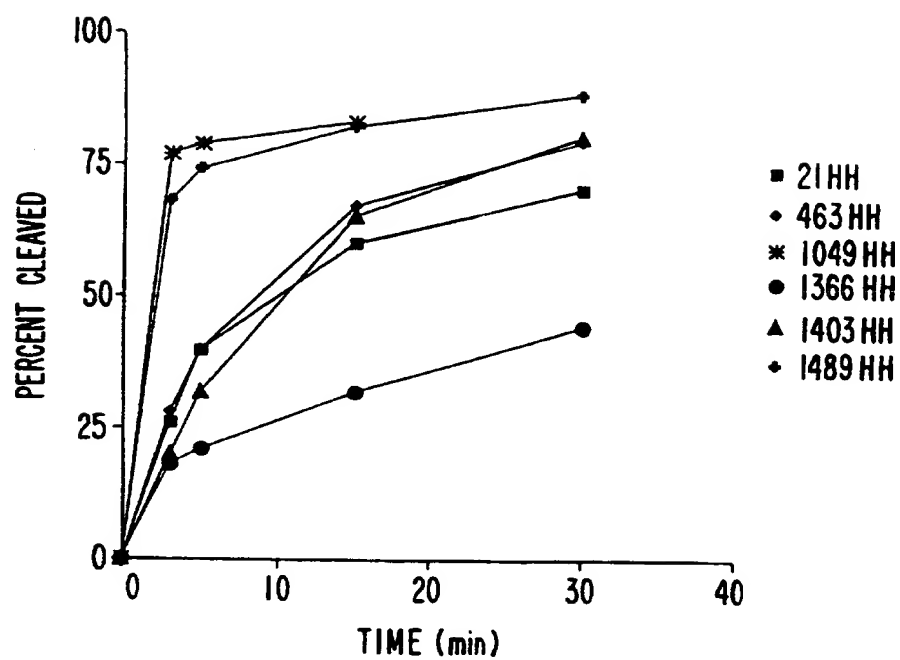


FIG. 7.

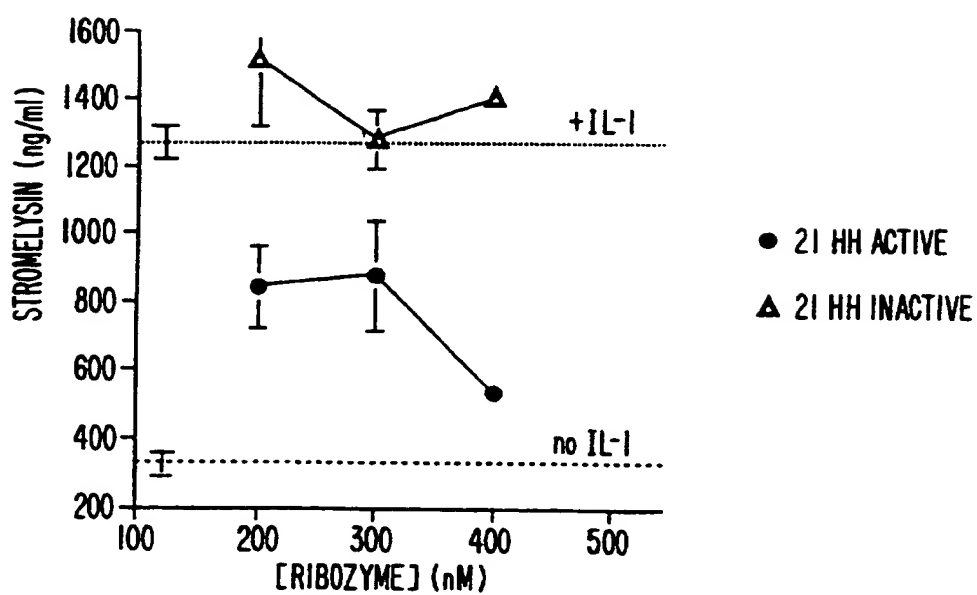


FIG. 8.

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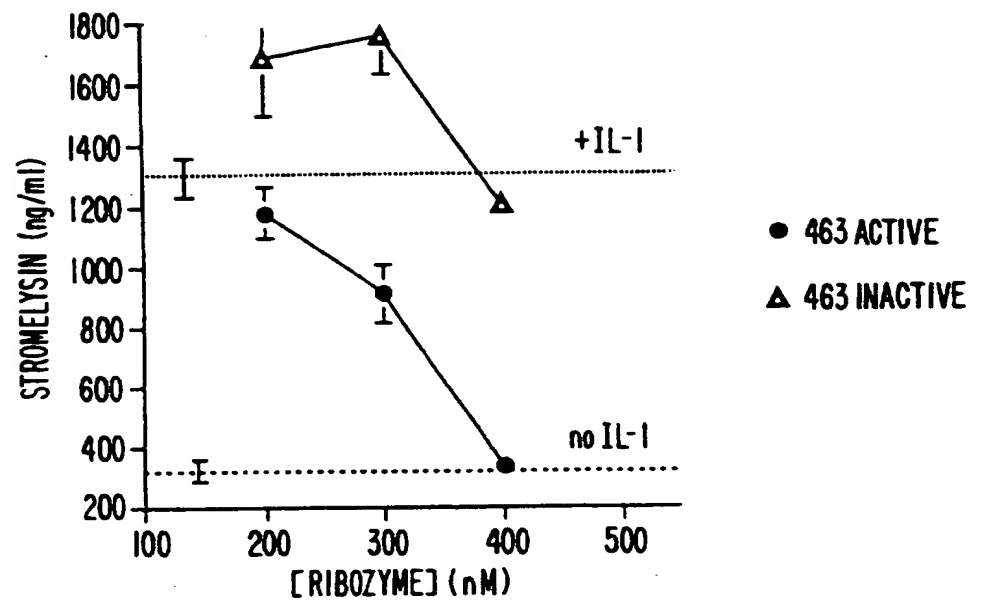


FIG. 9.

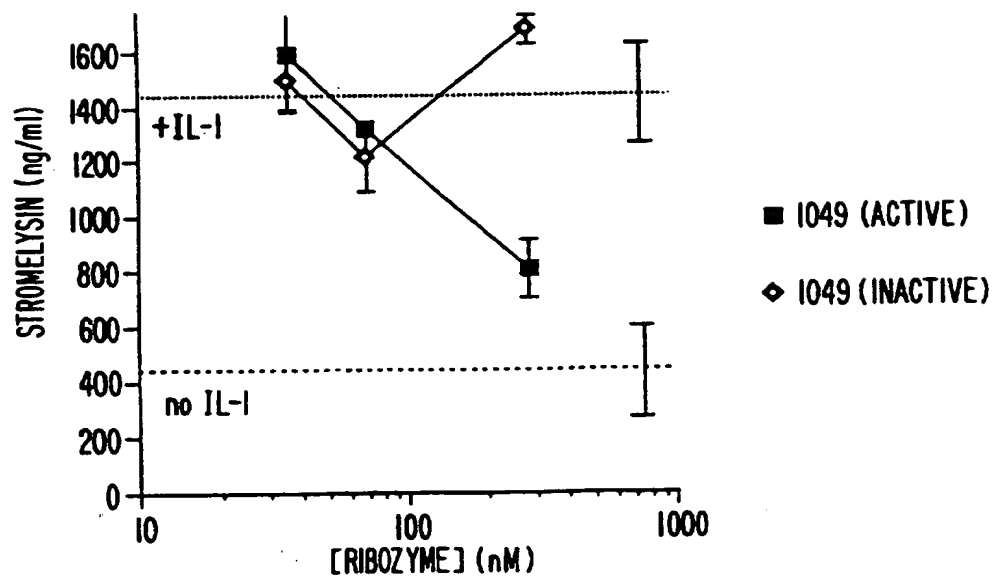


FIG. 10.

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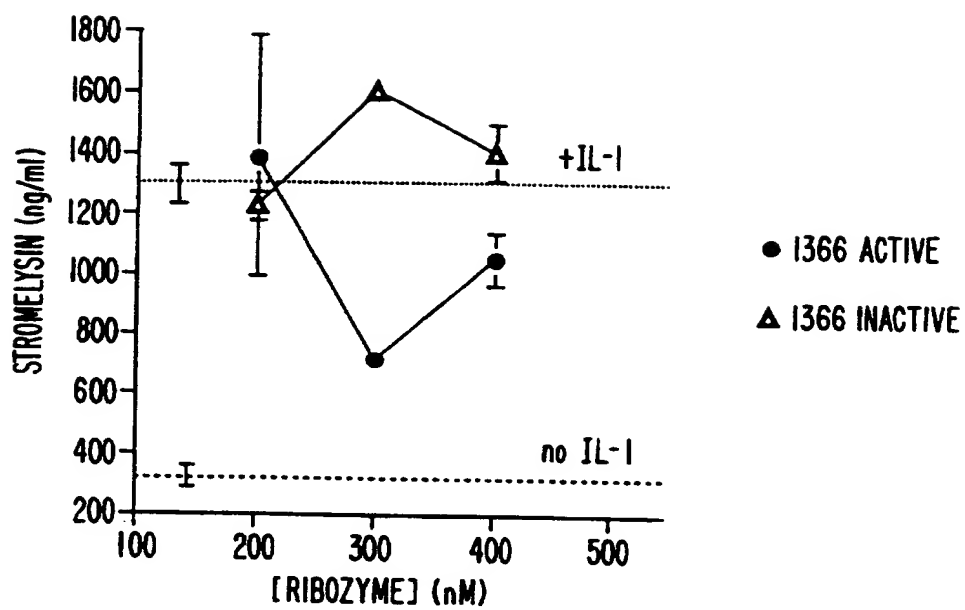


FIG. 11.

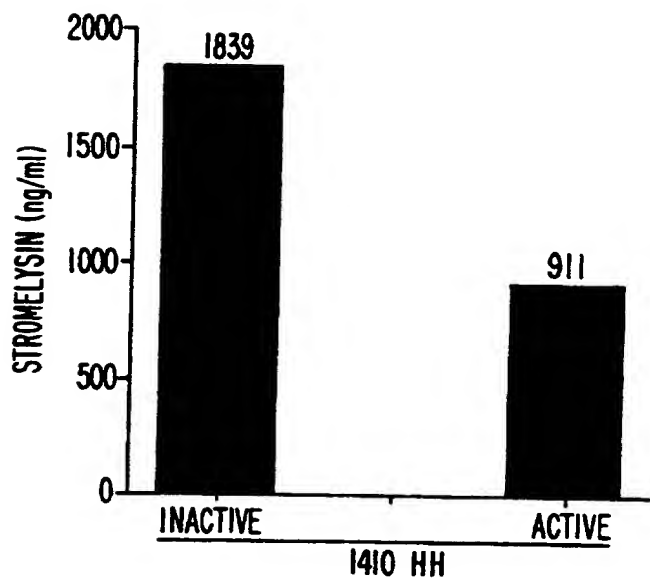


FIG. 12.

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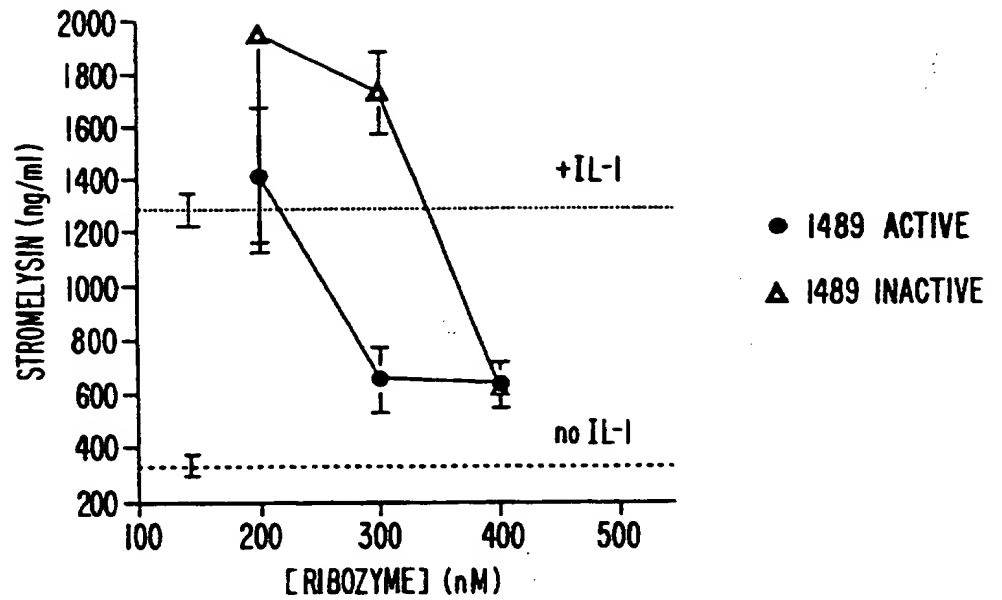


FIG. 13.

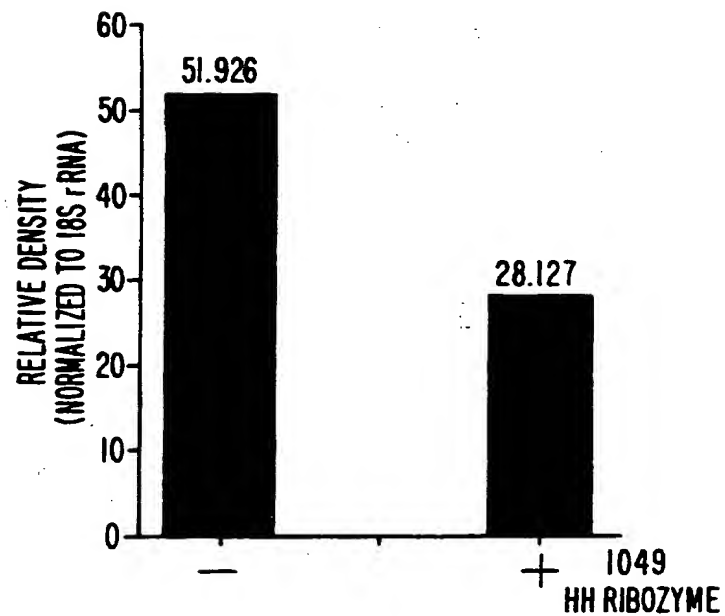


FIG. 14.

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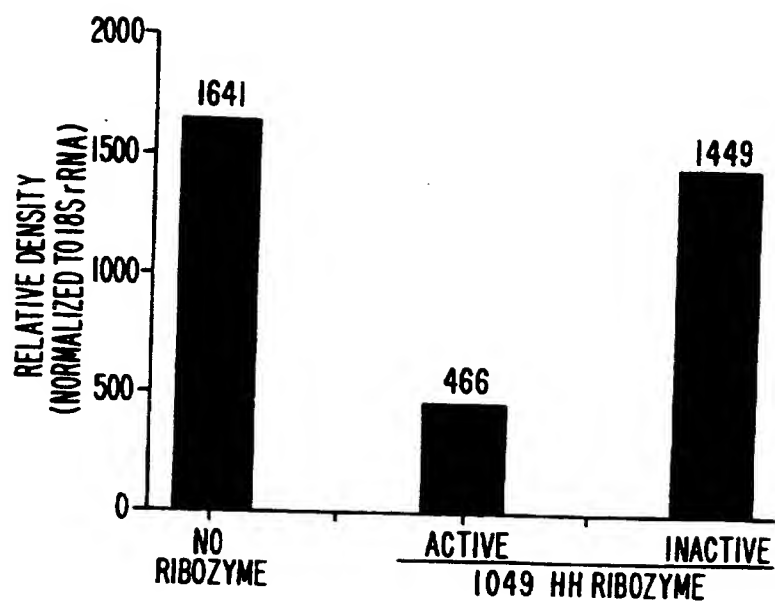


FIG. 15.

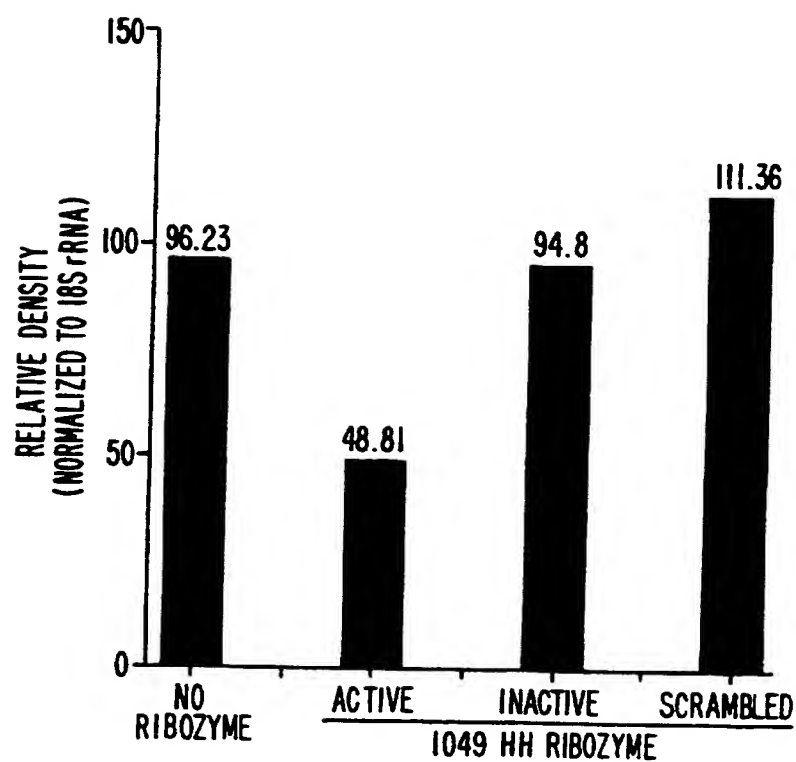
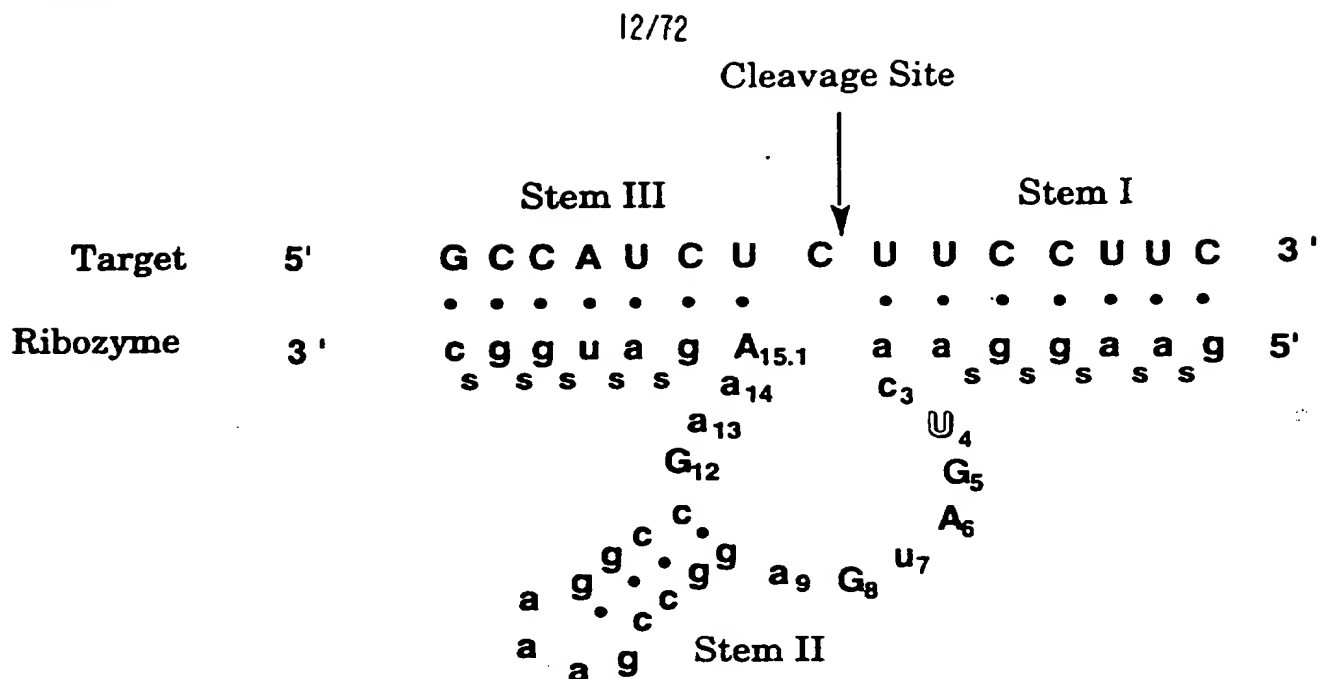


FIG. 16.

SUBSTITUTE SHEET (RULE 26)



Upper case= ribonucleotides

Lower case= 2'-O-methyl nucleotides

U = 2'-C-Allyl modification

s = phosphorothioate linkages

FIG. 17a.

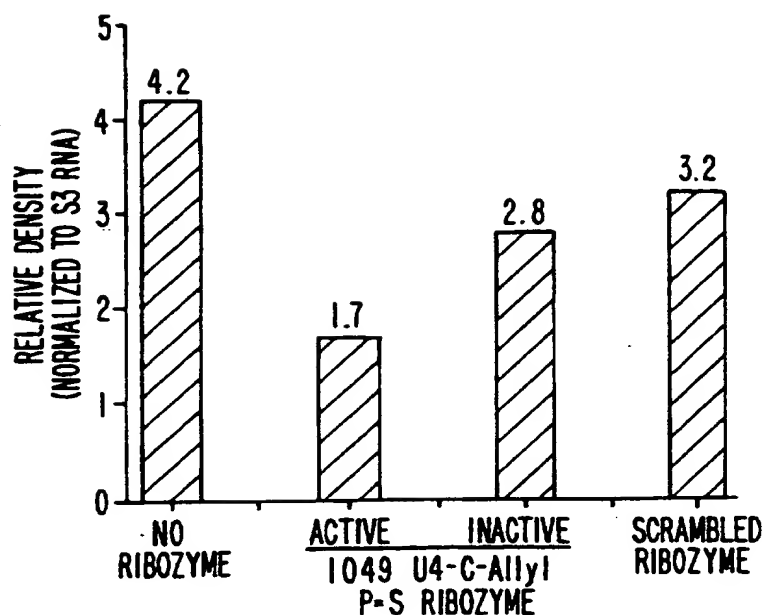


FIG. 17b.

SUBSTITUTE SHEET (RULE 26)

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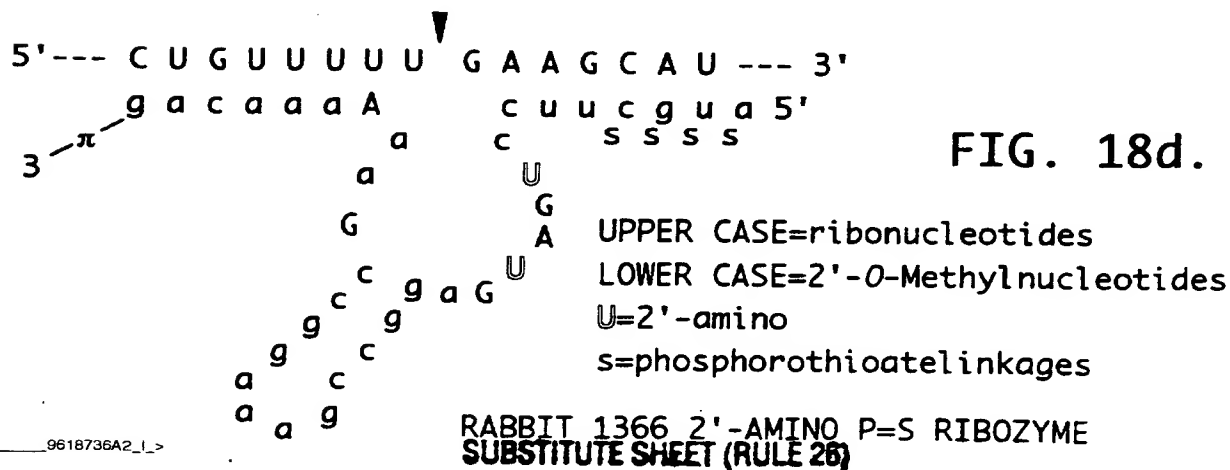
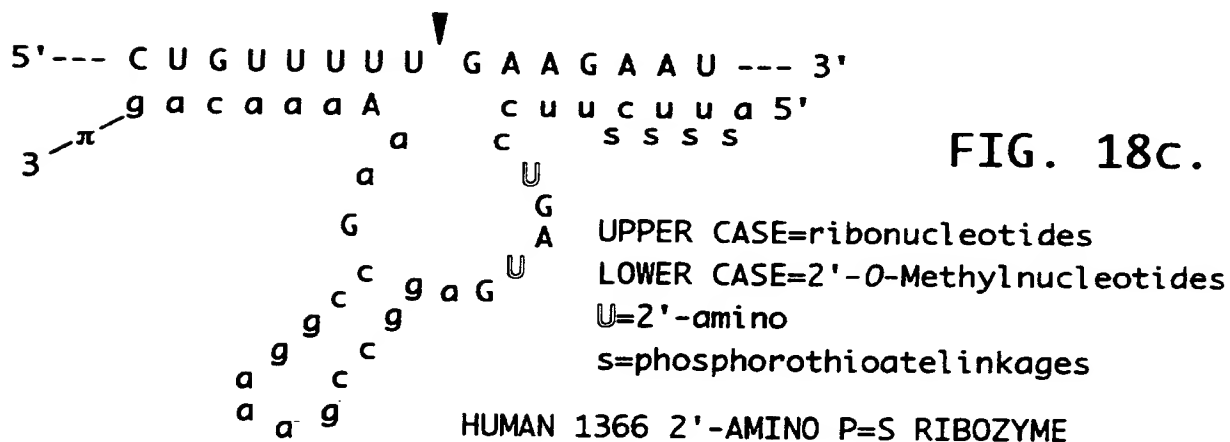
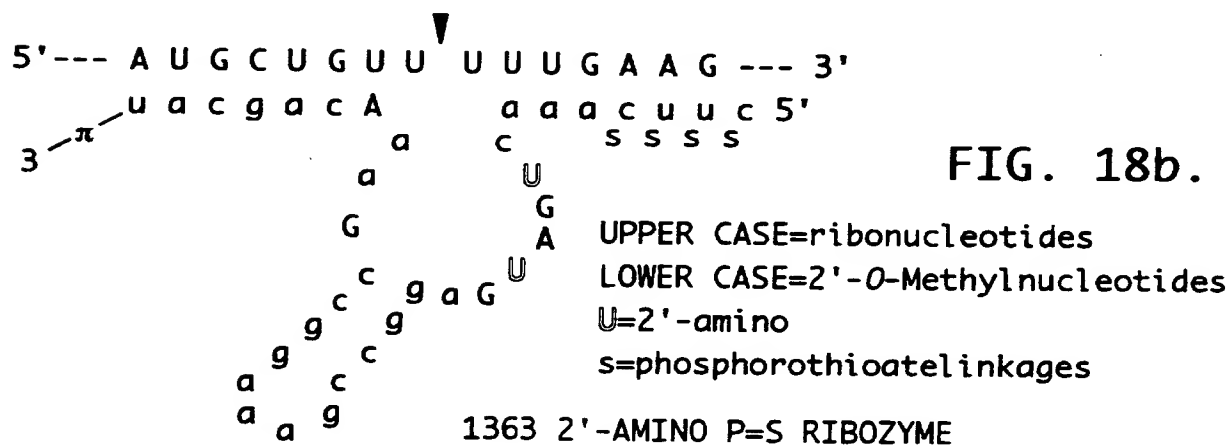
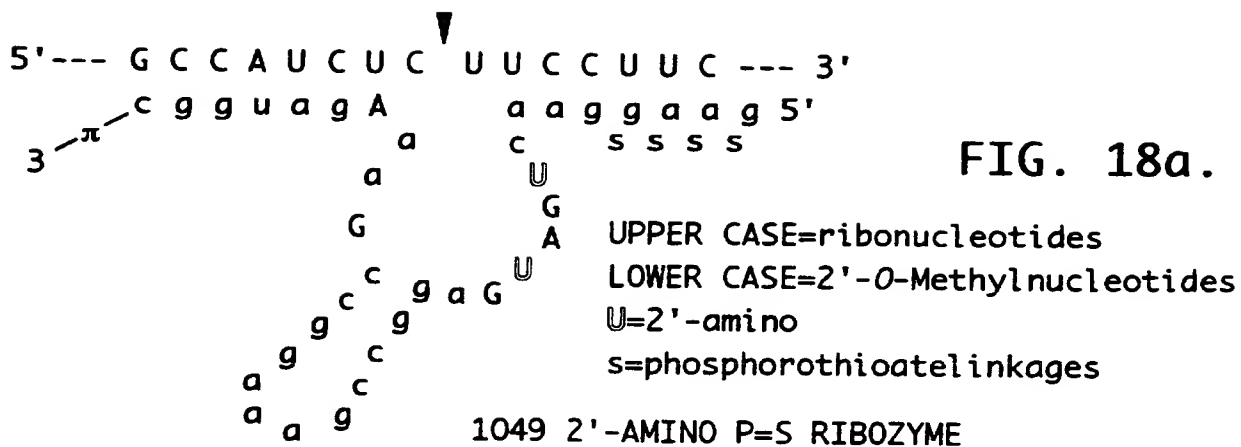


FIG. 19.

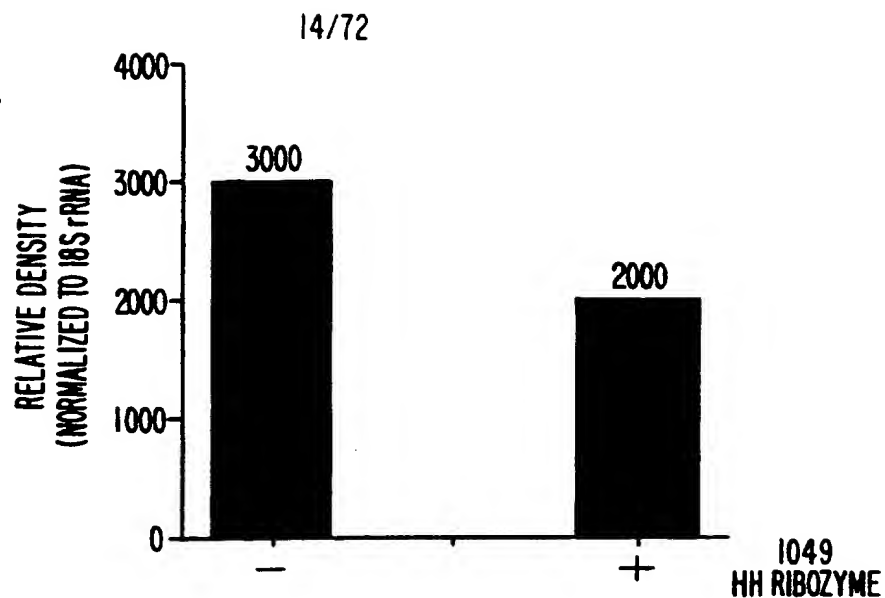


FIG. 20.

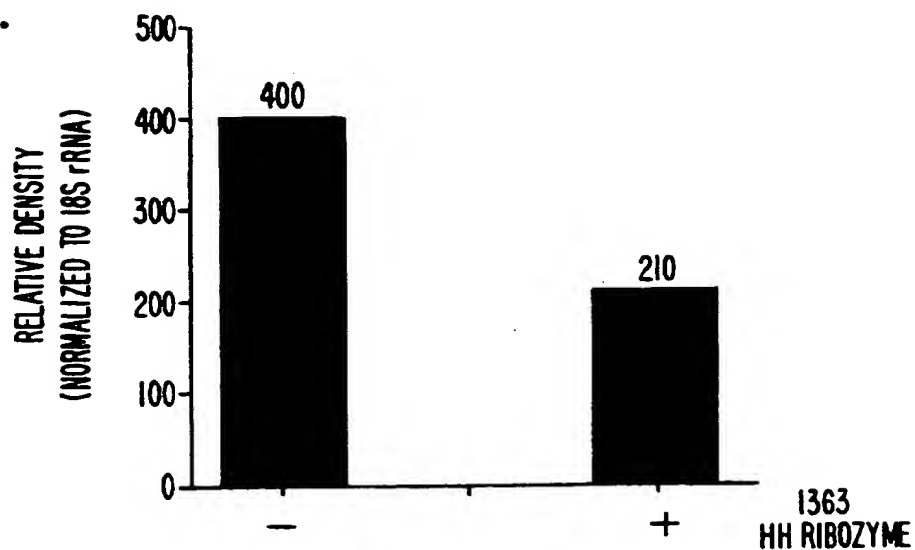
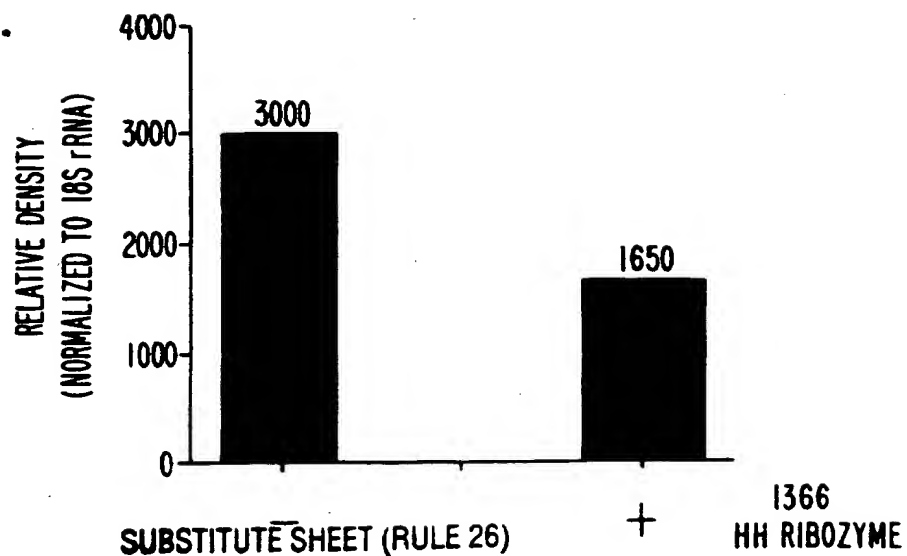
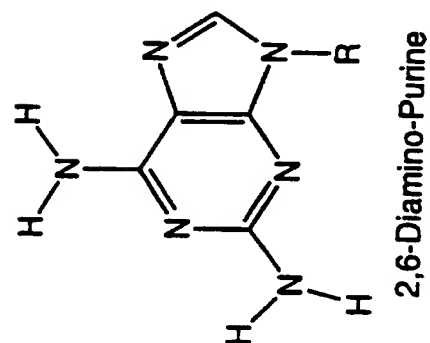
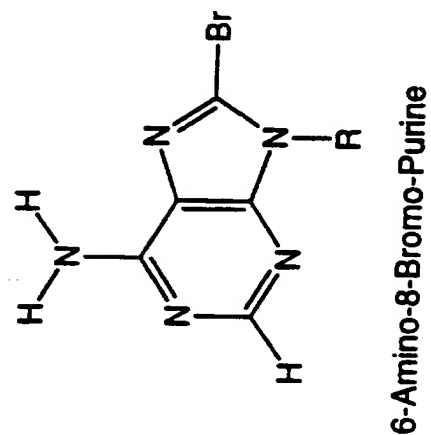
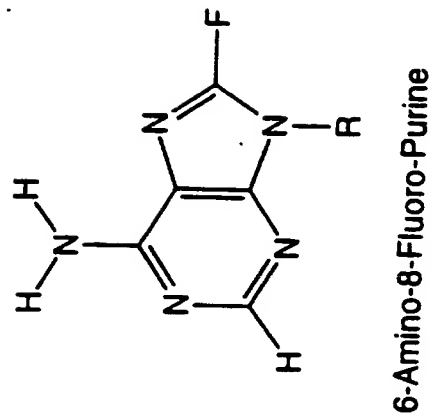
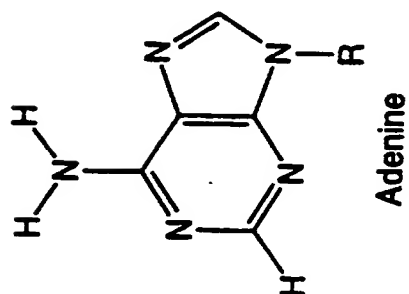
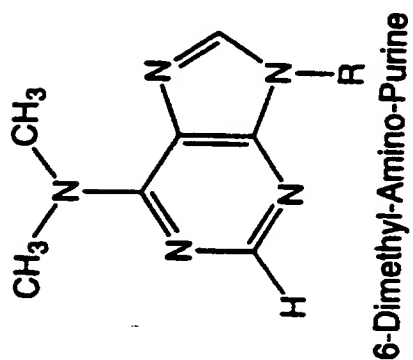
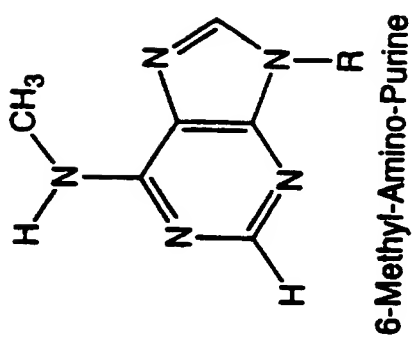
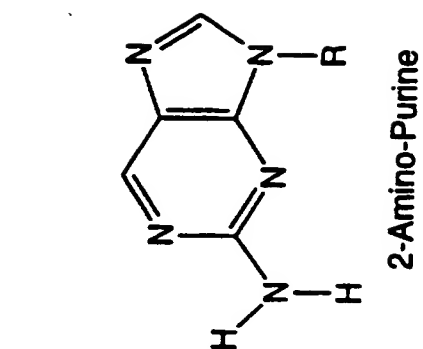


FIG. 21.



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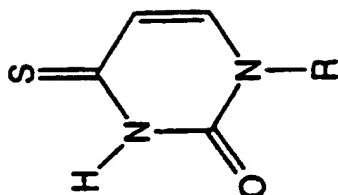
FIG. 22a.



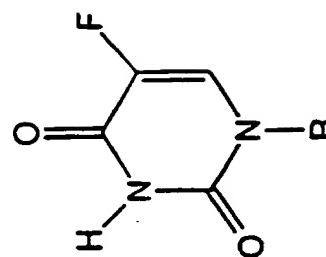
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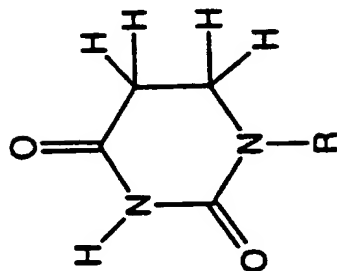
FIG. 22b.



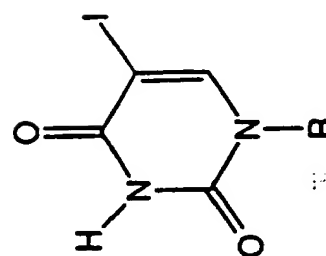
4-Thio-Uracil



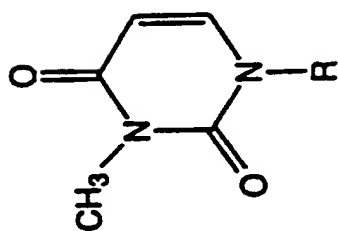
5-Fluoro-Uracil



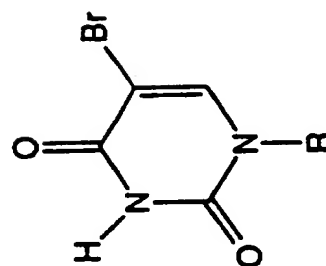
5,6-Dihydro-Uracil



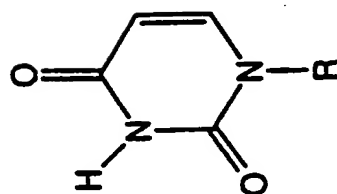
5-Iodo-Uracil



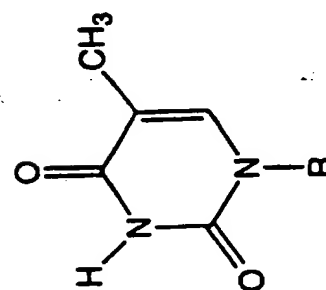
3-Methyl-Uracil



5-Bromo-Uracil



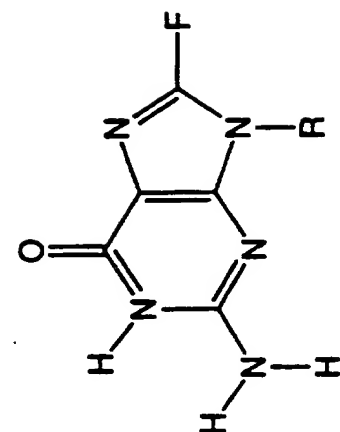
Uracil



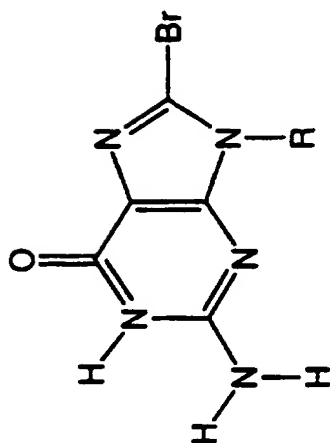
Thymine

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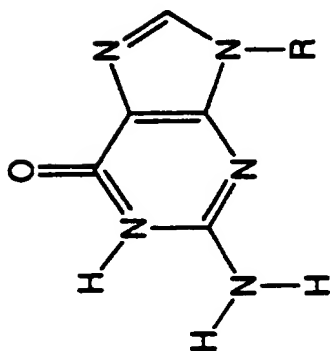
FIG. 22c.



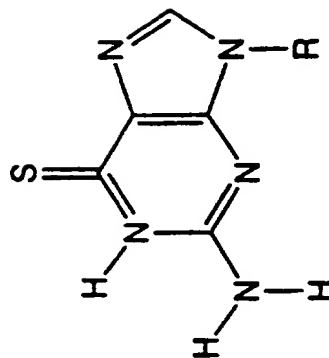
8-Fluoro-Guanine



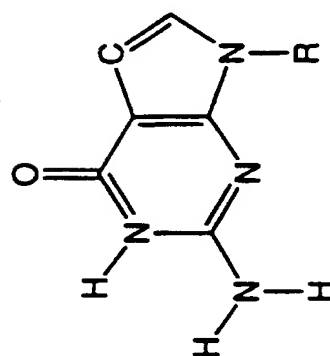
8-Bromo-Guanine



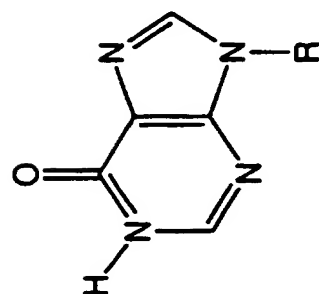
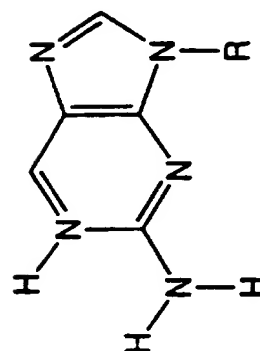
Guanine



6-Thio-Guanine



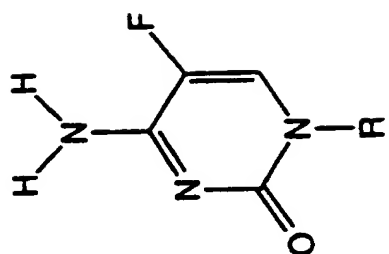
7-Deaza-Guanine

Hypoxanthine
(Inosine base)

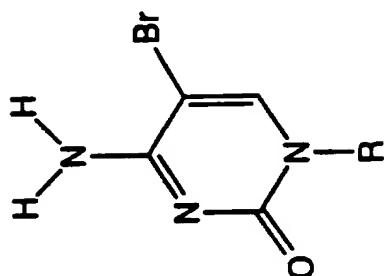
2-Amino-Purine

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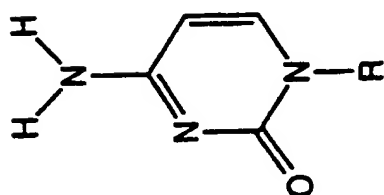
FIG. 22d.



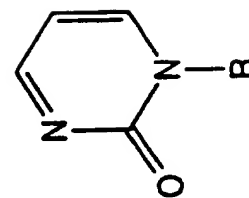
5-Fluoro-Cytosine



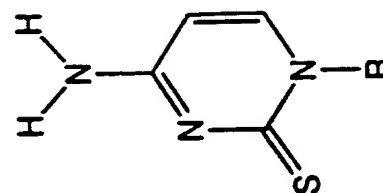
5-Bromo-Cytosine



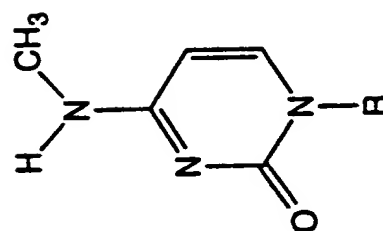
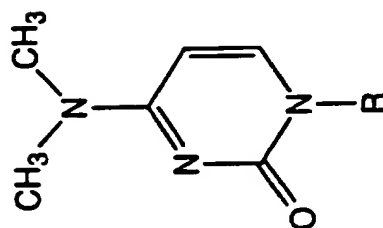
Cytosine



2-Pyridone



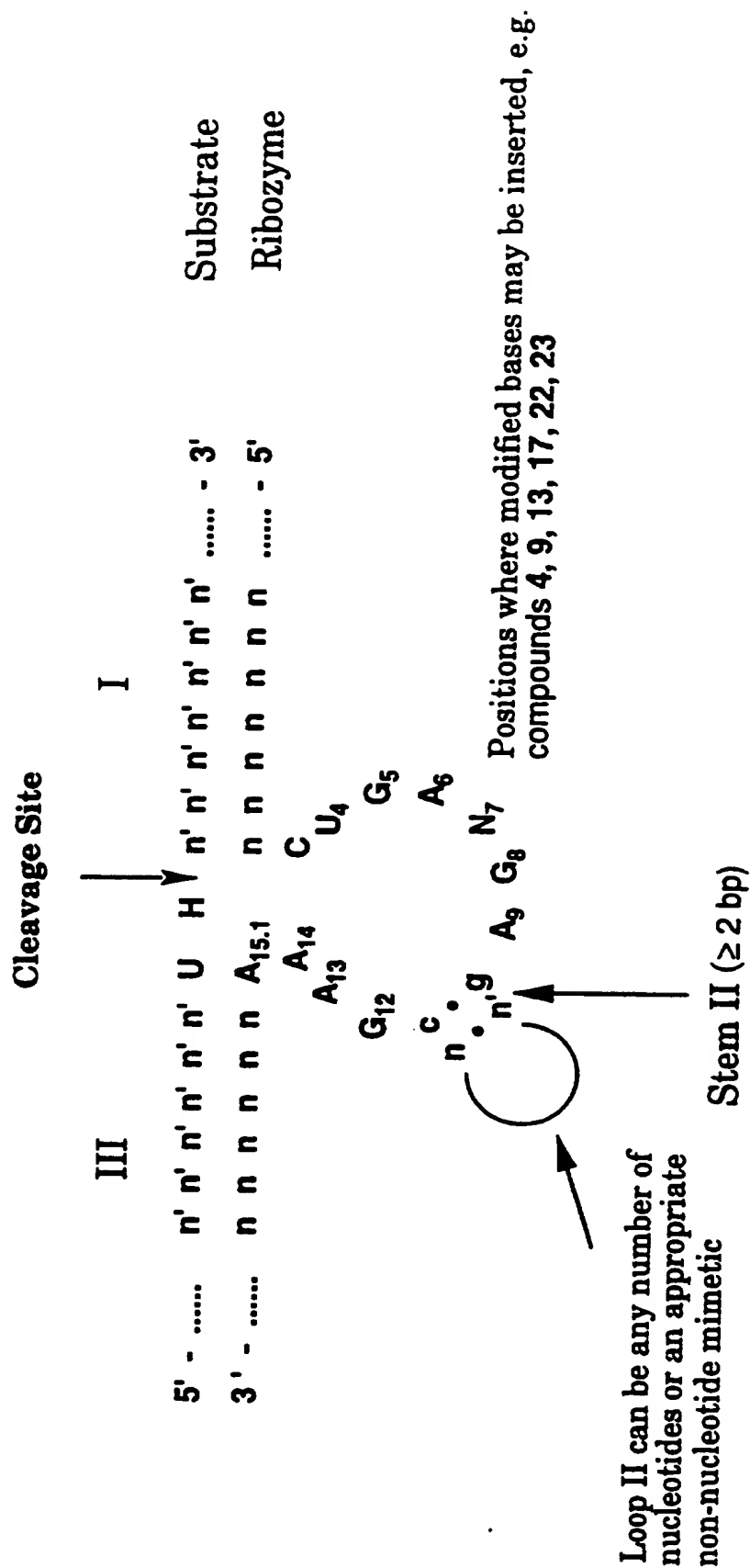
2-Thio-Cytosine

N⁴-Methyl-CytosineN⁴,N⁴-dimethyl-Cytosine

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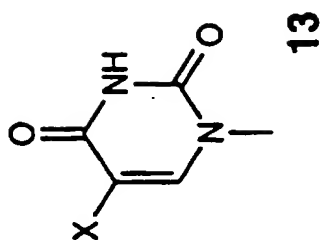
FIG. 23.



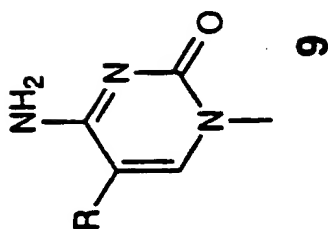
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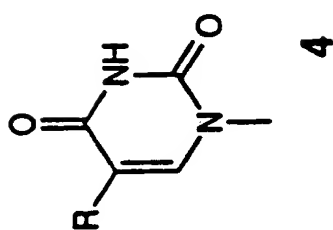
FIG. 24.



5-Halouridine

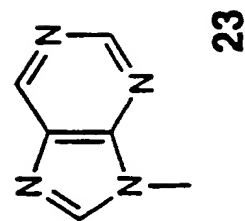
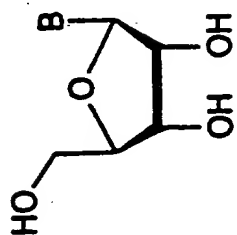


5-Alkylcytidine

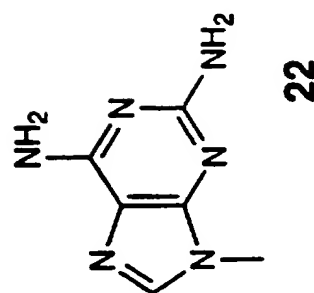


5-Alkyluridine

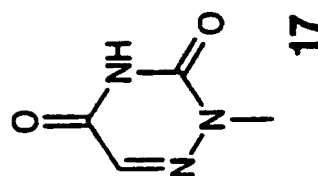
B =



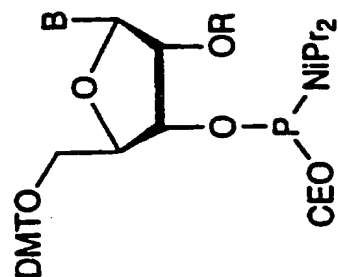
Purine



Diaminopurine



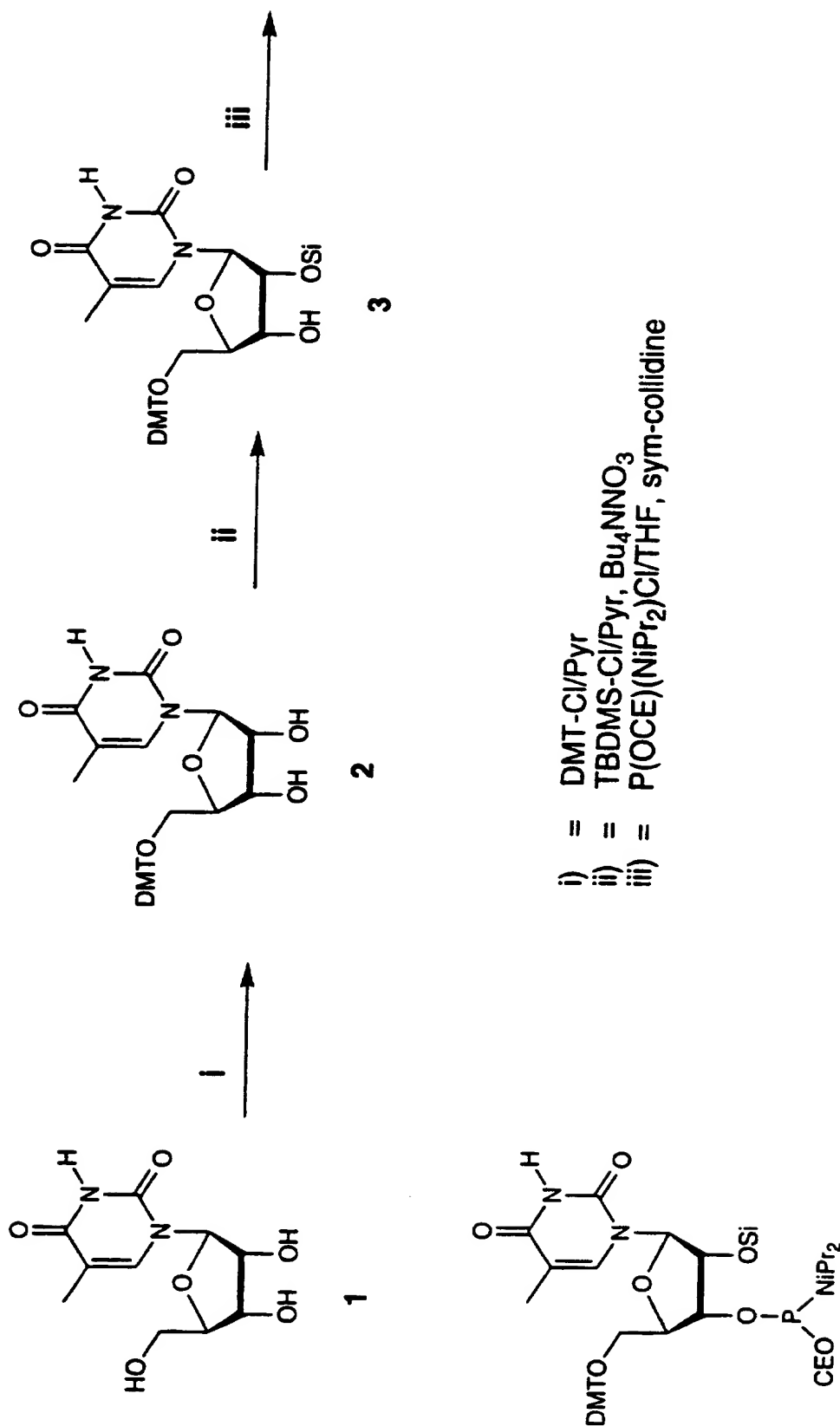
6-Azauridine



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FIG. 25.



SUBSTITUTE SHEET (RULE 26)

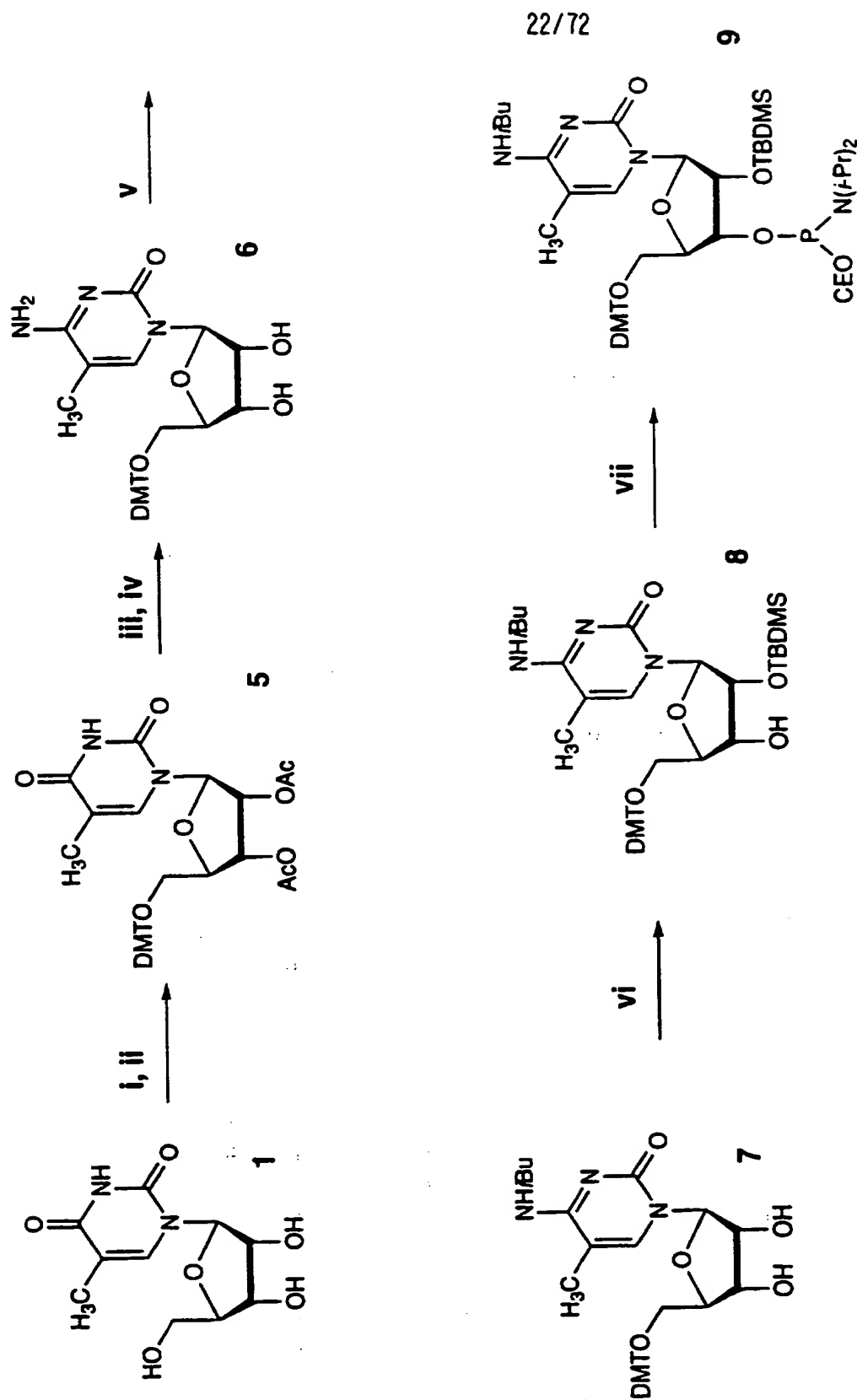
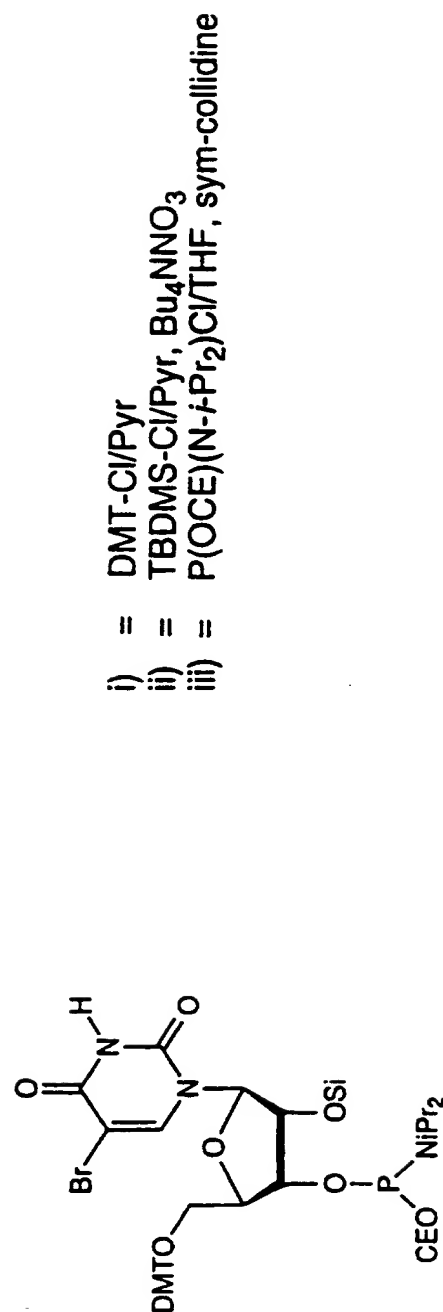
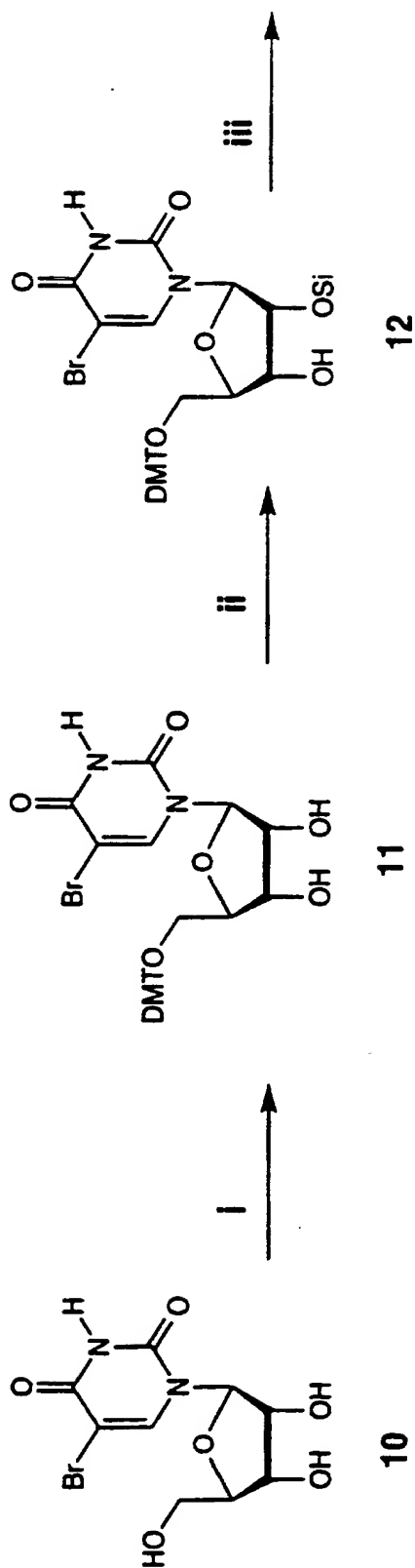


FIG. 26.

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FIG. 27.

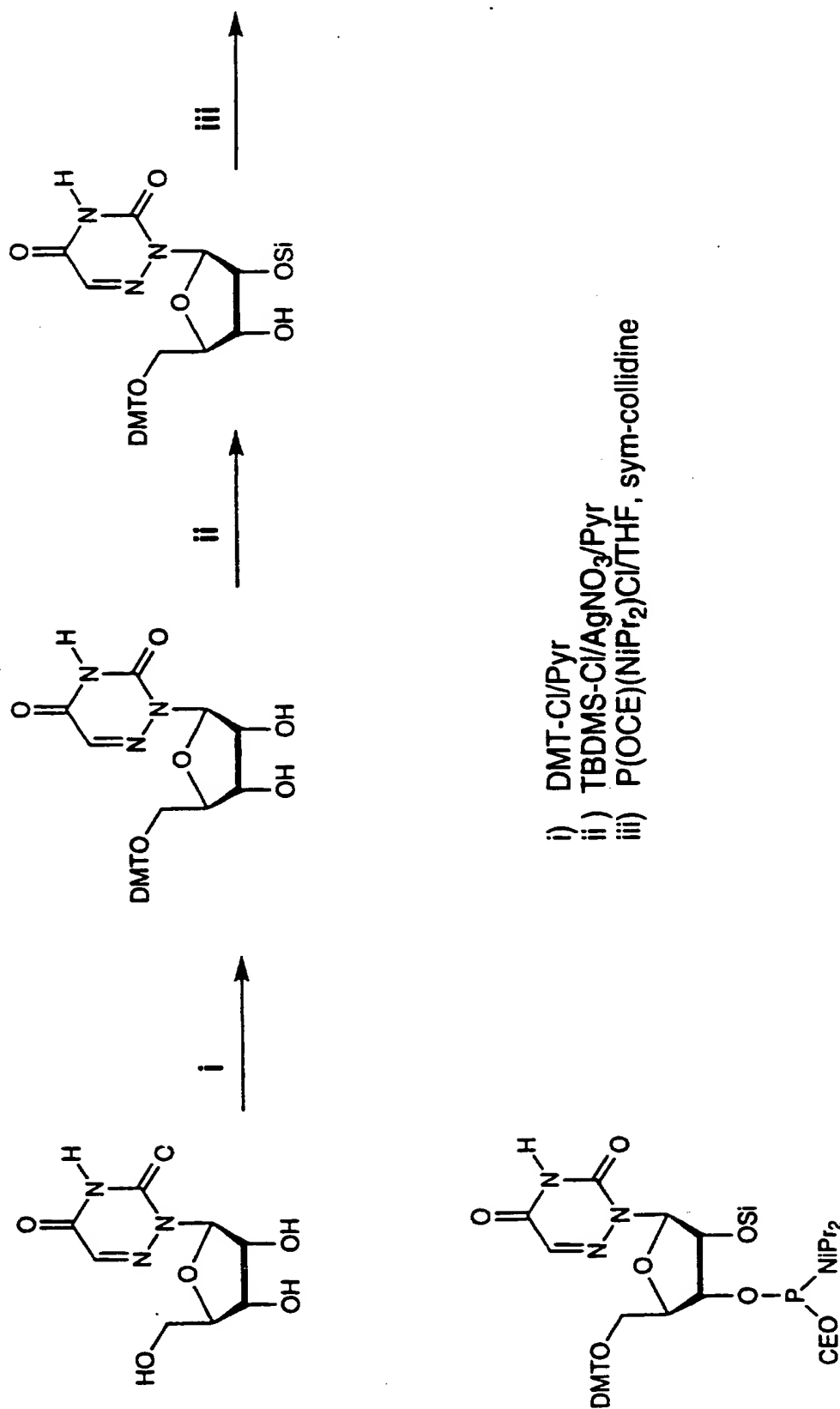


i) = DMT-Cl/Pyr
 ii) = TBDMS-Cl/Pyr, Bu₄NNO₃
 iii) = P(OCE)(N-*i*-Pr₂)Cl/THF, sym-collidine

SUBSTITUTE SHEET (RULE 26)

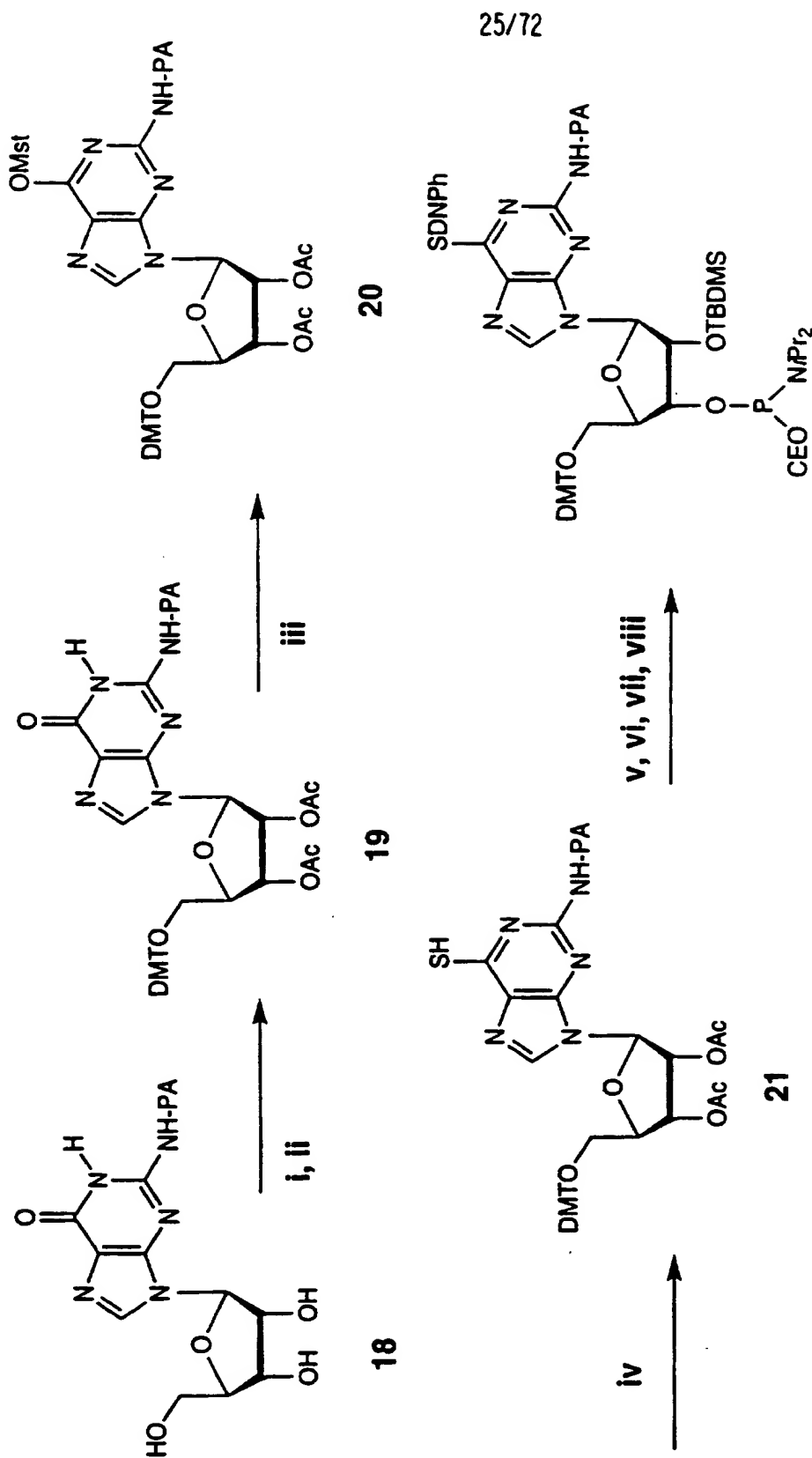
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FIG. 28.



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FIG. 29.

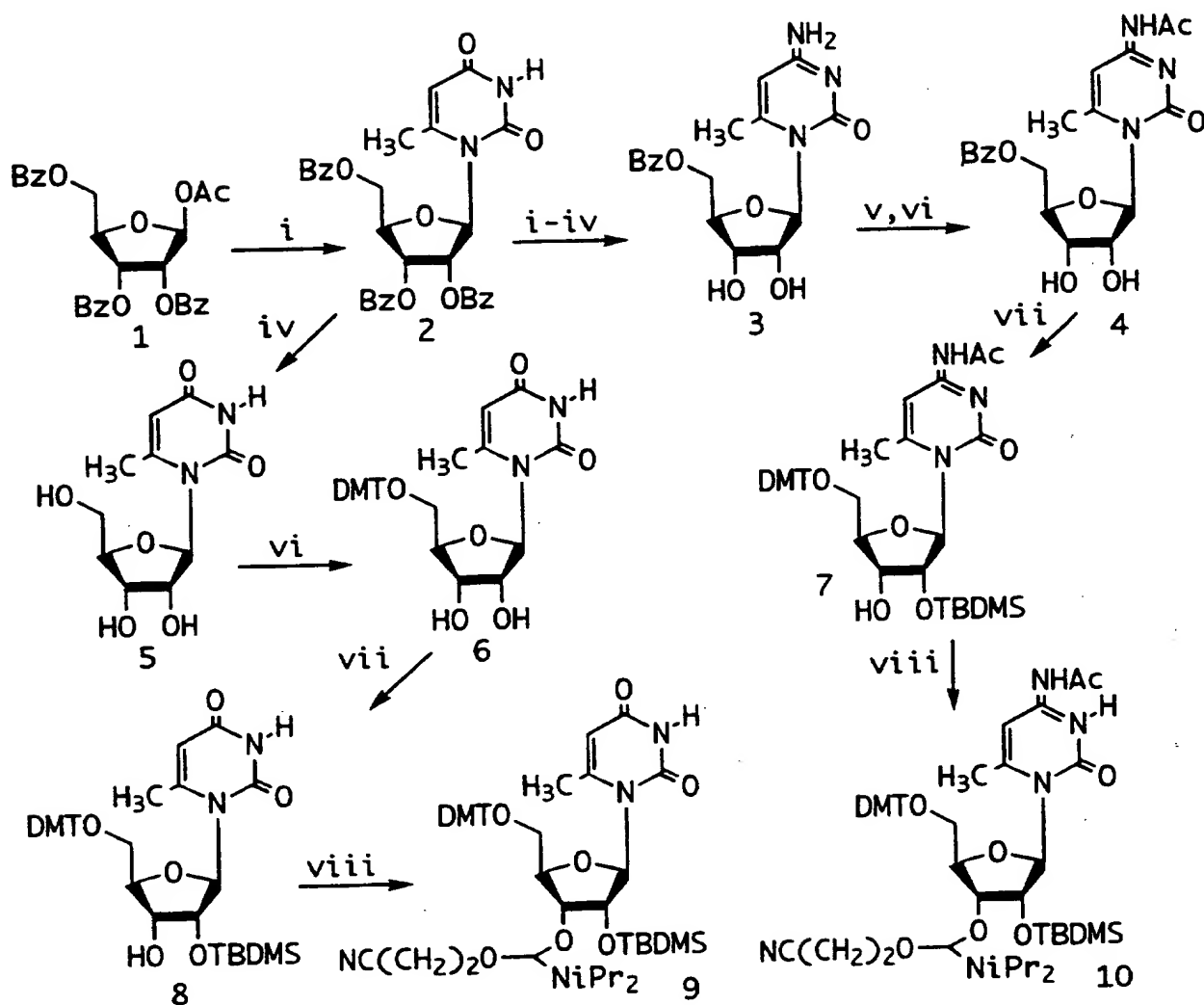


- i) DMT-Cl/Pyr
 ii) Ac₂O/Pyr
 iii) Mst-Cl
 iv) Li₂S/THF
- v) 1M NaOH/Pyr/MeOH
 ii) DNPh-F/MeCN
 iii) TBDMS-Cl/Pyr
 iv) P(OCE)(N-iPr₂)Cl

Mst = mesitylenesulfonyl
 DNPh =Dinitrophenyl

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REAGENTS AND CONDITIONS:

- i) 6-Me-Ura^{TMS}, CF₃SO₃SIME₃, 0°C;
- ii) 1,2,4-triazole, POCl₃; iii) NH₄OH/dioxane;
- iv) 2M NaOH/Pyr/MeOH; v) MeSI-CL/PYR, THEN AC₂O;
- vi) DMT-Cl/Pyr;
- vii) TBDMS-Cl/AgNO₃/Pyr/THF;
- viii) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, DIPEA/CH₂Cl₂.

FIG. 30.

SUBSTITUTE SHEET (RULE 26)

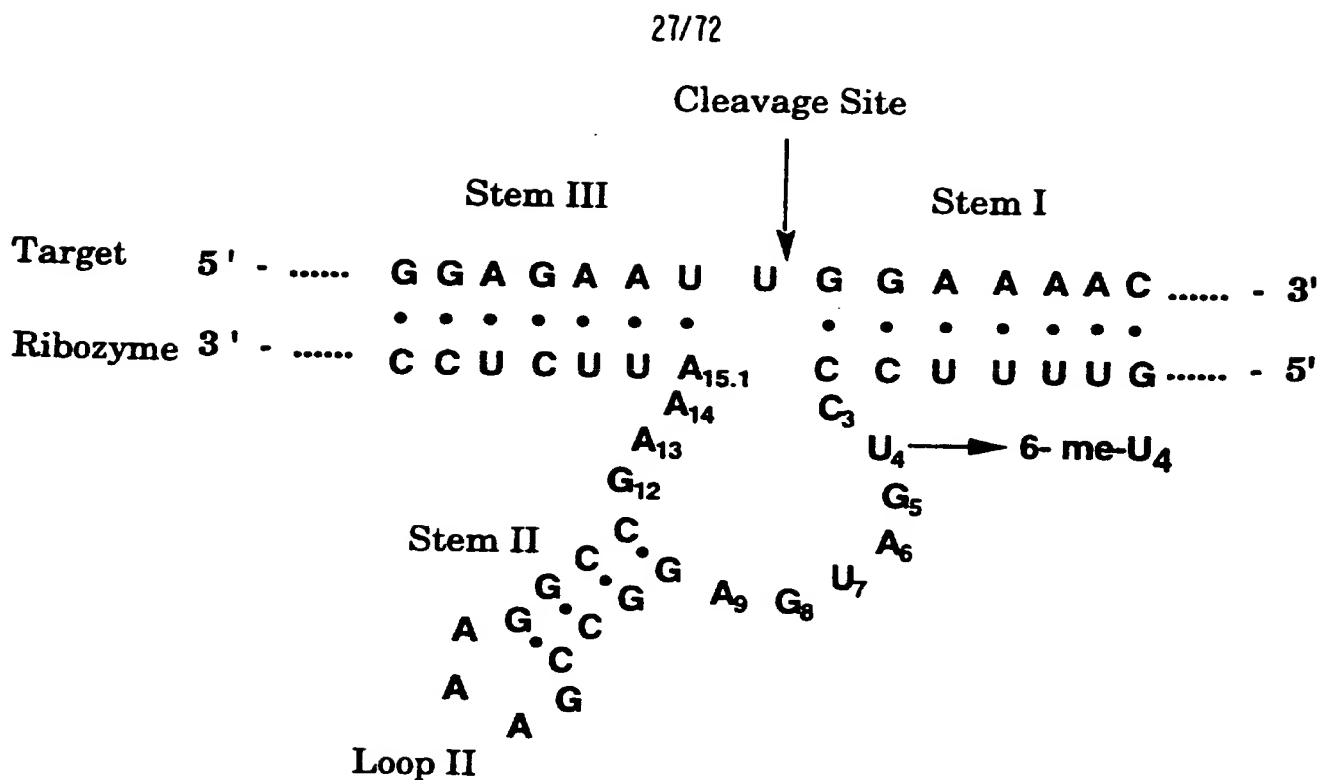
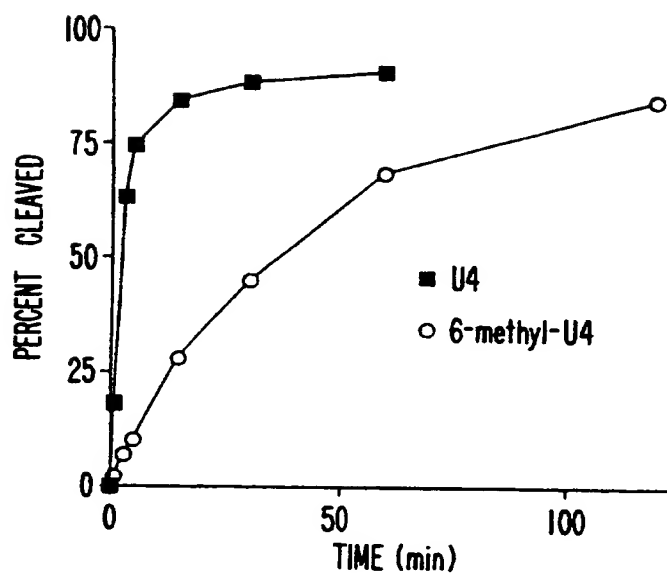


FIG. 31.



[Ribozyme]=40nM [Substrate]=~1nM

FIG. 32.

SUBSTITUTE SHEET (RULE 26)

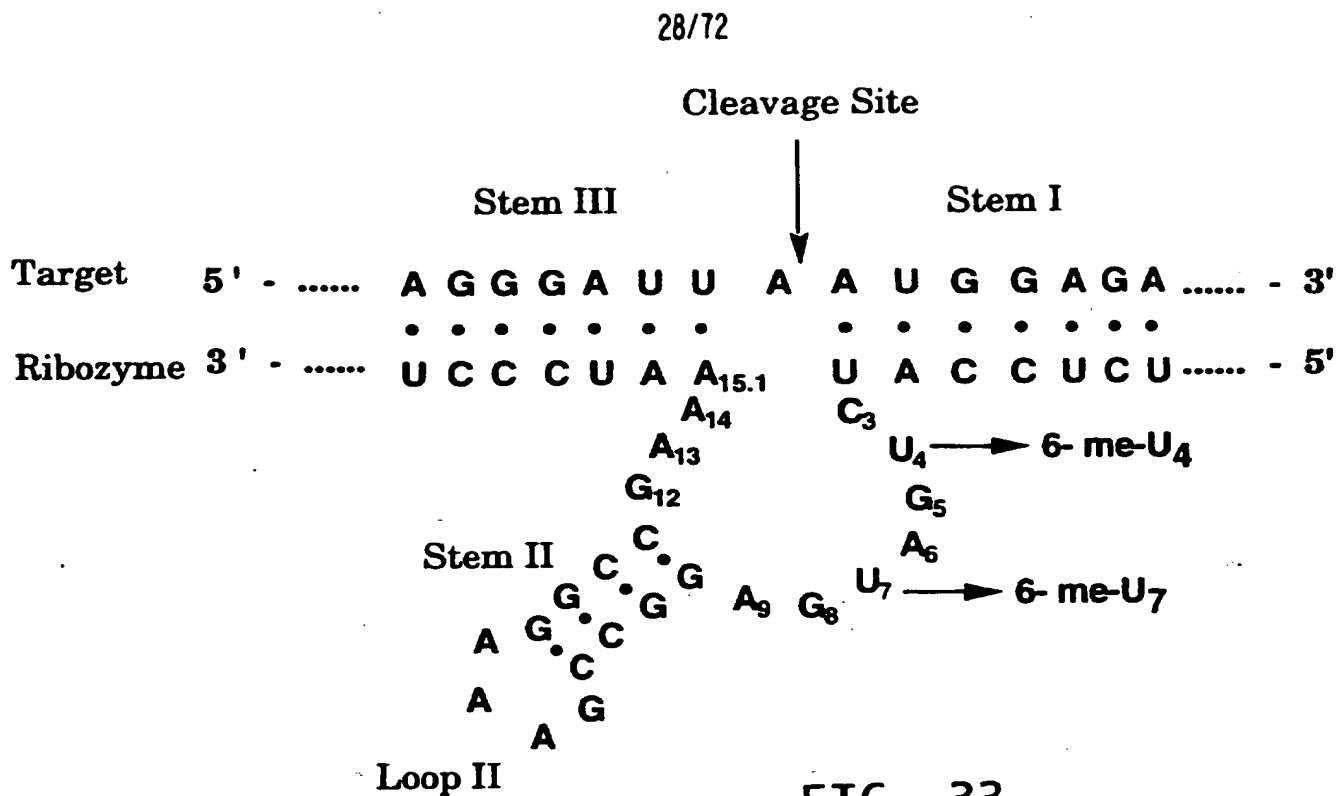
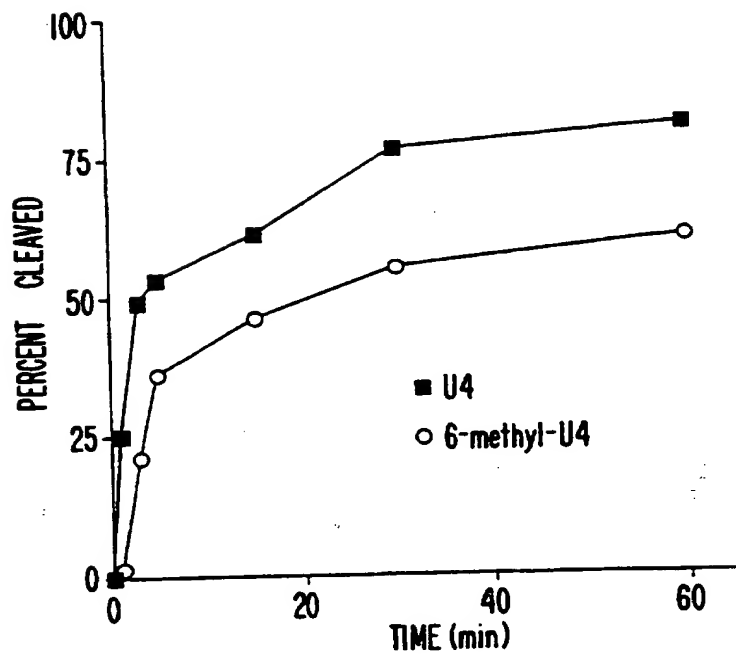


FIG. 33.

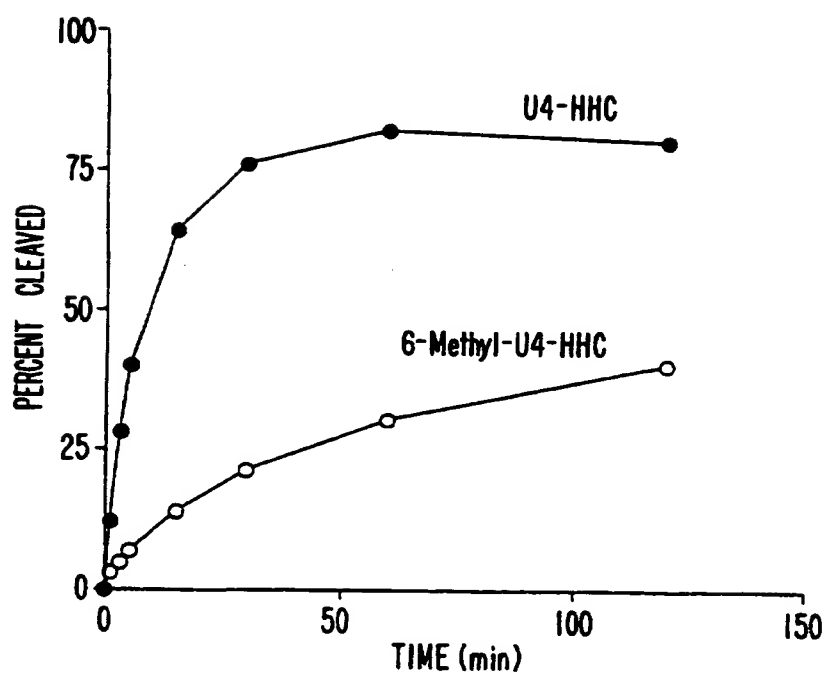
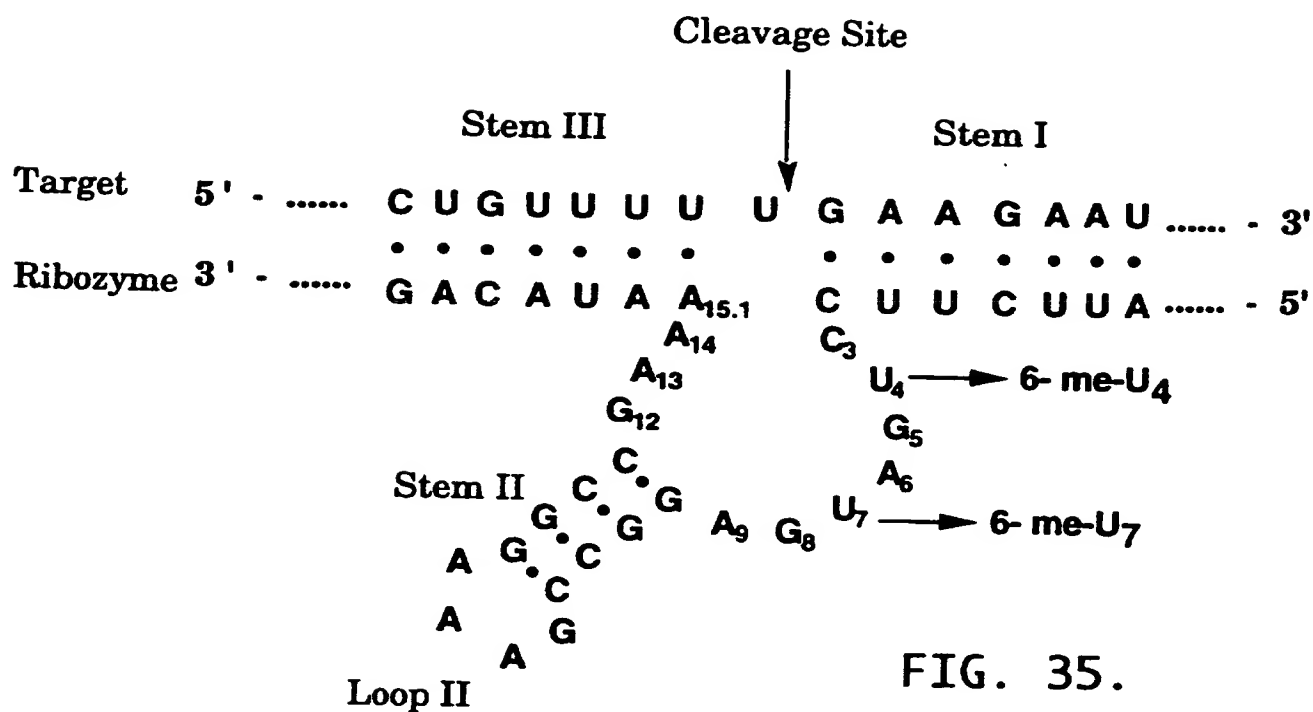


[Ribozyme]=40nM [Substrate]=~1nM

FIG. 34.

SUBSTITUTE SHEET (RULE 26)

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[Ribozyme]=40nM [Substrate]=~1nM

FIG. 36.
SUBSTITUTE SHEET (RULE 26)

FIG. 37.

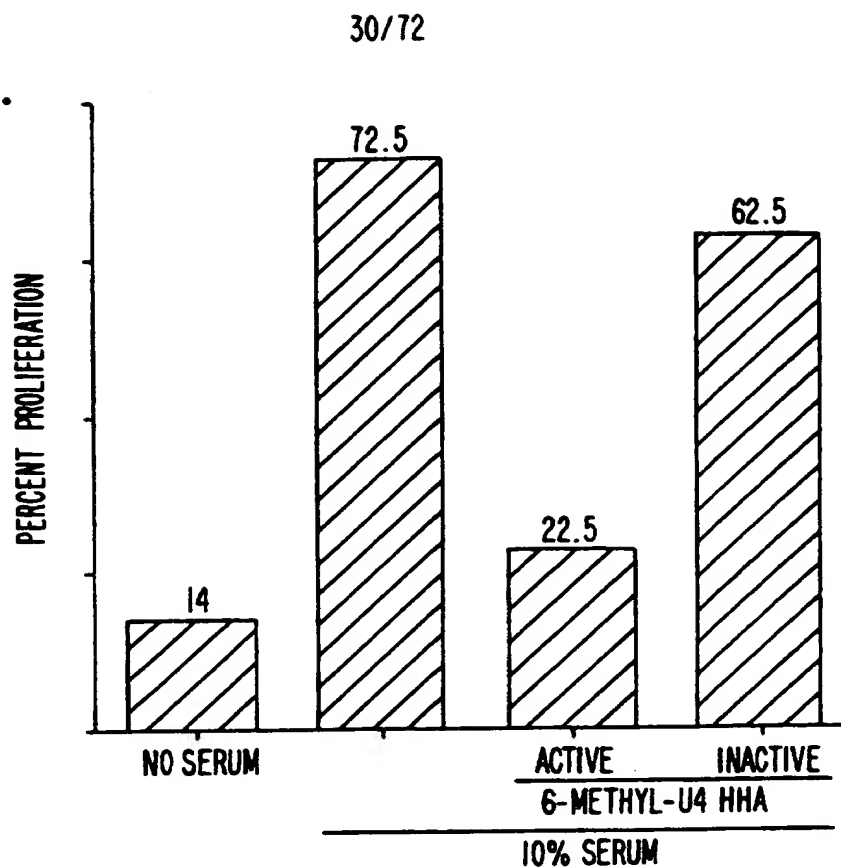
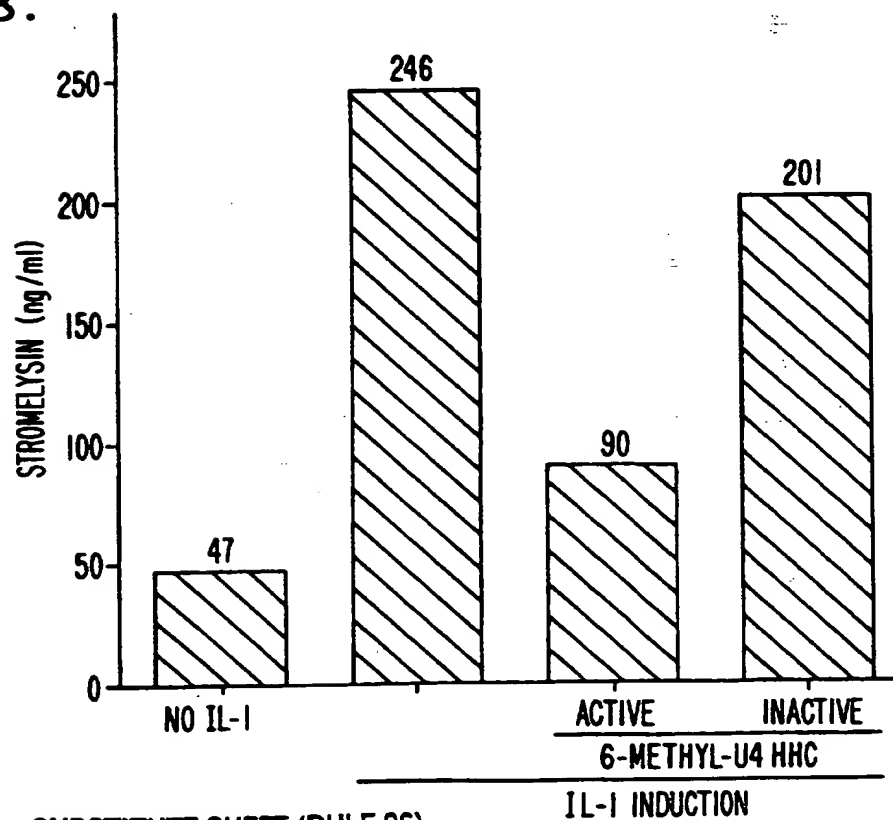
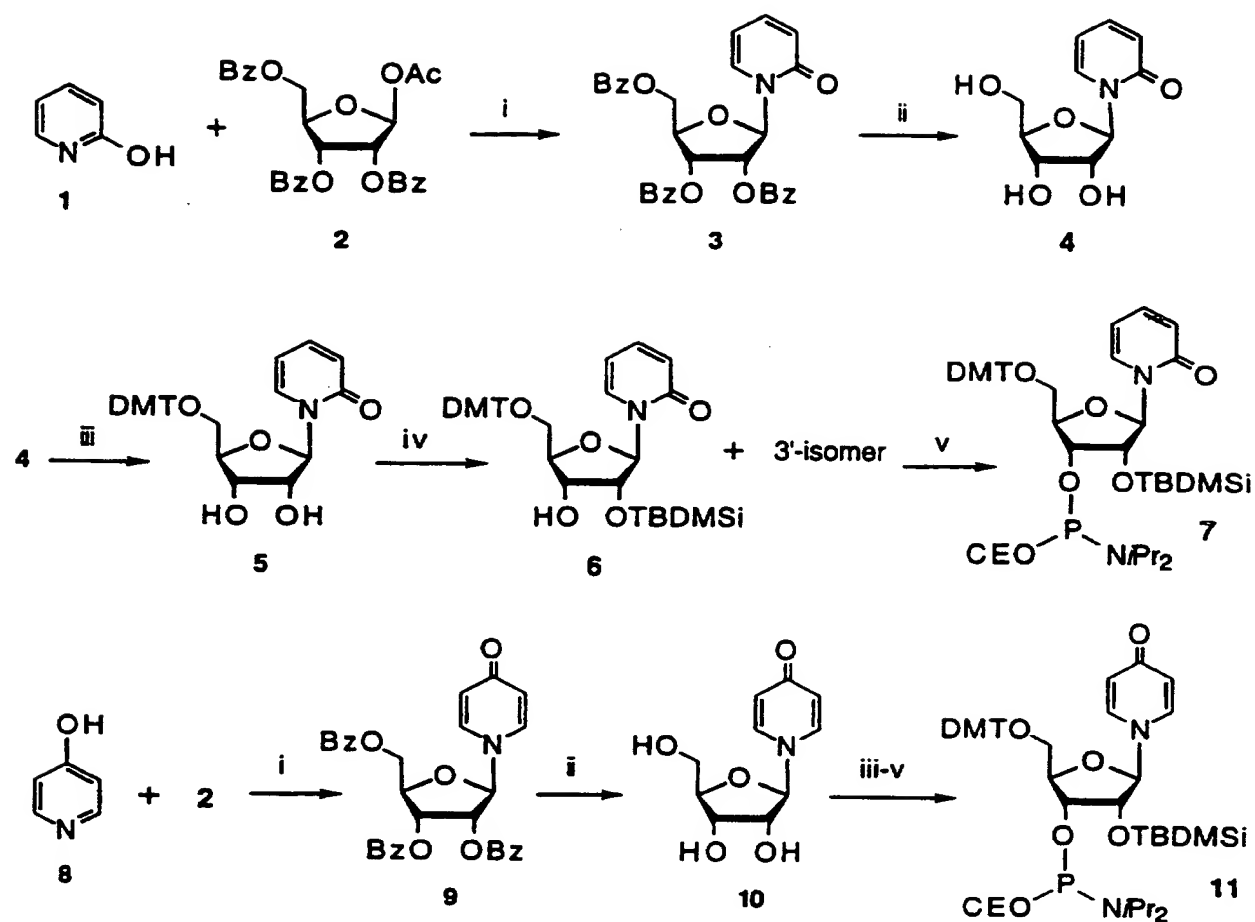


FIG. 38.



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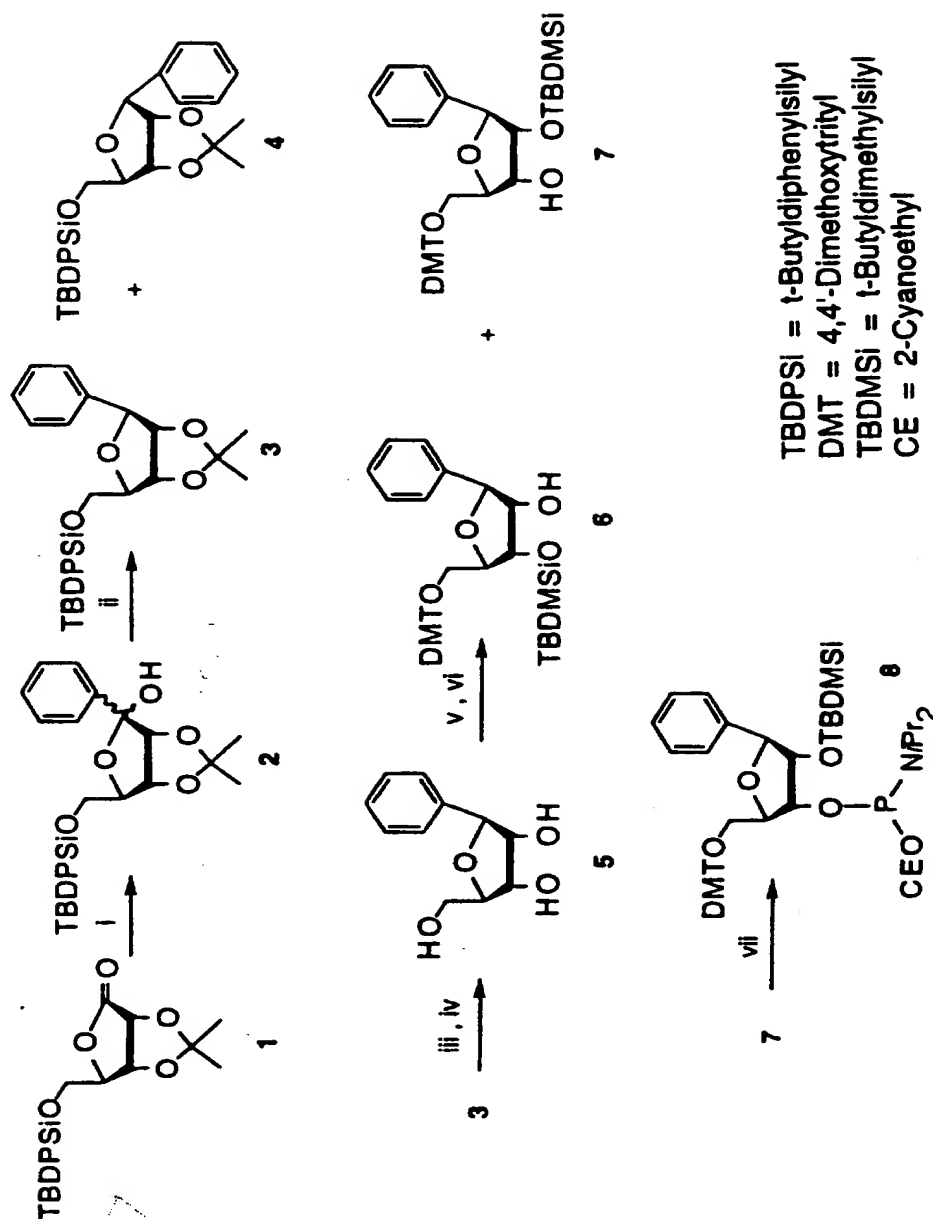


Bz = Benzoyl
 Ac = Acetyl
 DMT = 4,4'-Dimethoxytrityl
 TBDMSi = *t*-Butyldimethylsilyl
 CE = 2-cyanoethyl

Reagents and Conditions: i: N,O-bis(trimethylsilyl)acetamide (BSA)/TMSTf/
 CH₃CN, 70 °C, ii: NaOCH₃/CH₃OH, iii: DMT-Cl/DMAP/Et₃N/Pyr, iv: TBDMSi-
 Cl/AgNO₃/Pyr/THF, v: P(OCE)(N-*i*Pr₂)Cl/DIPEA/1-MeIm/CH₂Cl₂.

FIG. 39.

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Reagents and Conditions: i: PhLi/THF, -78 °C, ii: Et₃SiH/BF₃·Et₂O/CH₃CN, -40 °C,
 iii: 1M TBAF/THF, iv: 70 % aq. CH₃COOH, 100 °C, v: DMT-Cl/DMAPEA/Et₃N/Pyr, vi:
 TBDMSI-Cl/AgNO₃/Pyr/THF, vii: P(CEO)(N-iPr₂)Cl/DIPEA/1-Melm/CH₂Cl₂.

FIG. 40.

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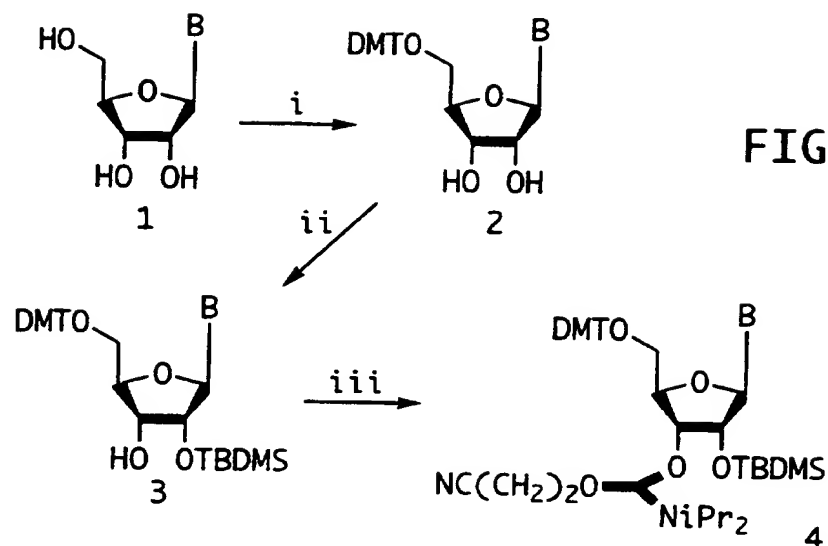


FIG. 41.

B=Pseudo U,2,4,6-trimethoxy benzene or 3-methyl U

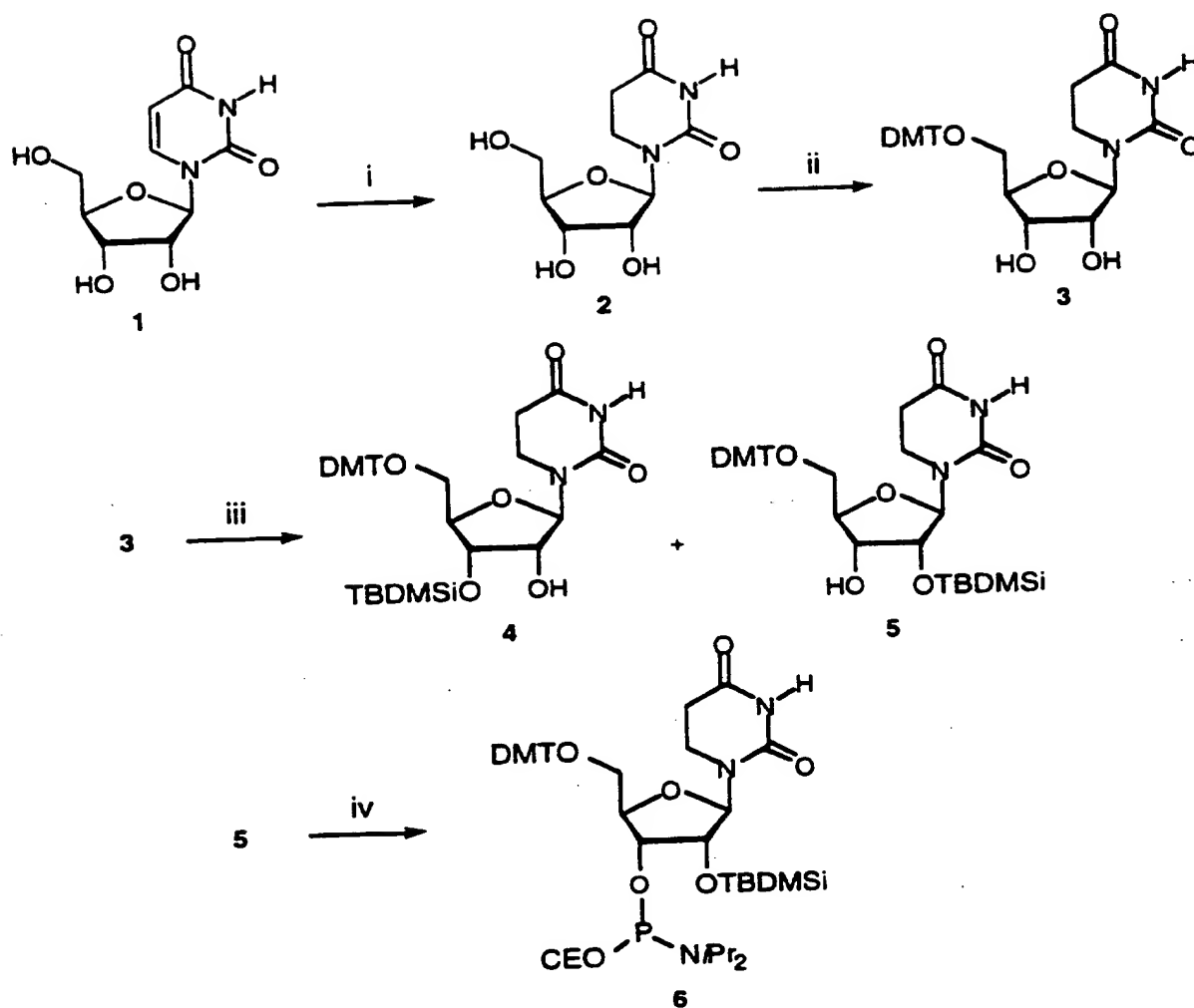
REAGENTS AND CONDITIONS:

- i) DMT-Cl/Pyr;
- ii) TBDMS-Cl/AgNO₃/Pyr/THF;
- iii) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, DIPEA/CH₂Cl₂.

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FIG. 42.



DMT = 4,4'-Dimethoxytrityl
 TBDMSi = t-Butyldimethylsilyl
 CE = 2-Cyanoethyl

Reagents and Conditions: i: Pd/Rh, H₂ 60 psi, ii: DMT-Cl/DMAP/Et₃N/Pyr,
 iii: TBDMSi-Cl/AgNO₃/Pyr/THF, iv: P(OCE)(N-iPr₂)Cl/DIPEA/1-Melm/CH₂Cl₂

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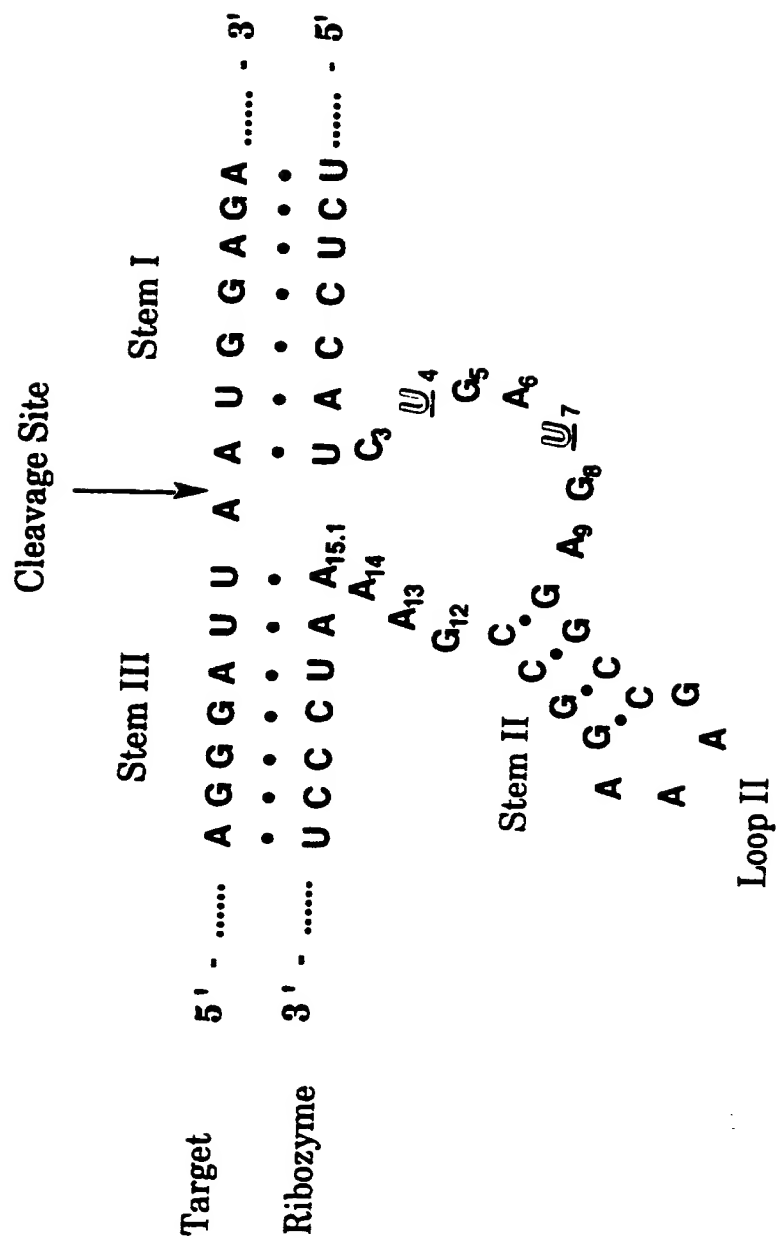
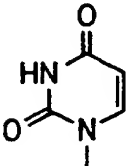
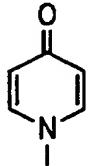
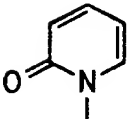
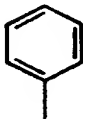
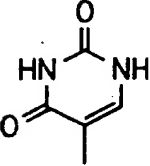
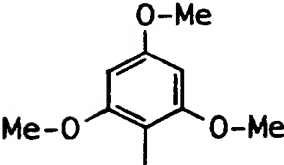
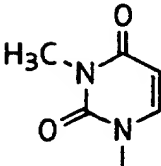


FIG. 43a.

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FIG. 43b.

	BASE MODIFICATIONS	HH nt. POSITION	
		<u>4</u>	<u>7</u>
		<u>k_{obs}(min⁻¹)</u>	
	U	2.1	2.1
	Pyridin-4-one	0.04	≥10
	Pyridin-2-one	0.03	1.2
	Phenyl	0.05	2.5
	Pseudo U	1.0	0.22
	3-O-Methoxy Benzene	0.02	0.14
	3-Methyl U	0.02	4.6

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FIG. 44a.

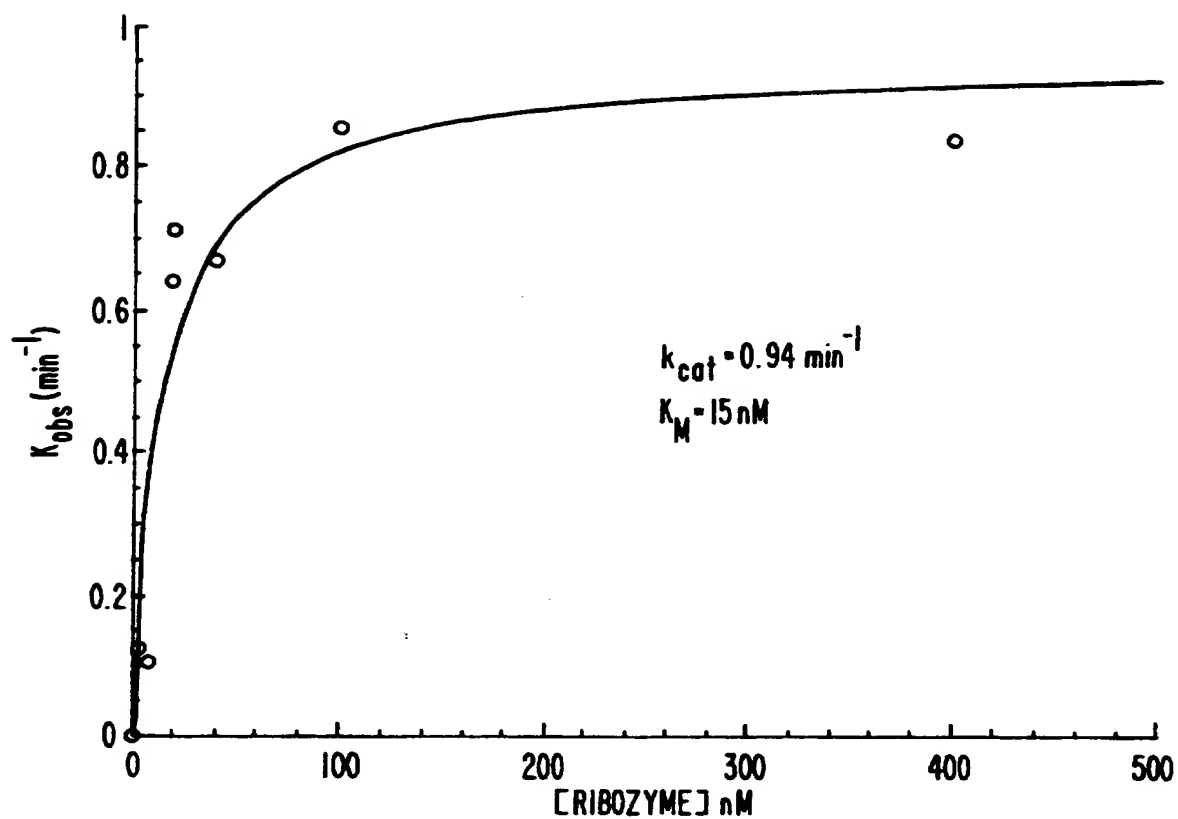


FIG. 44b.

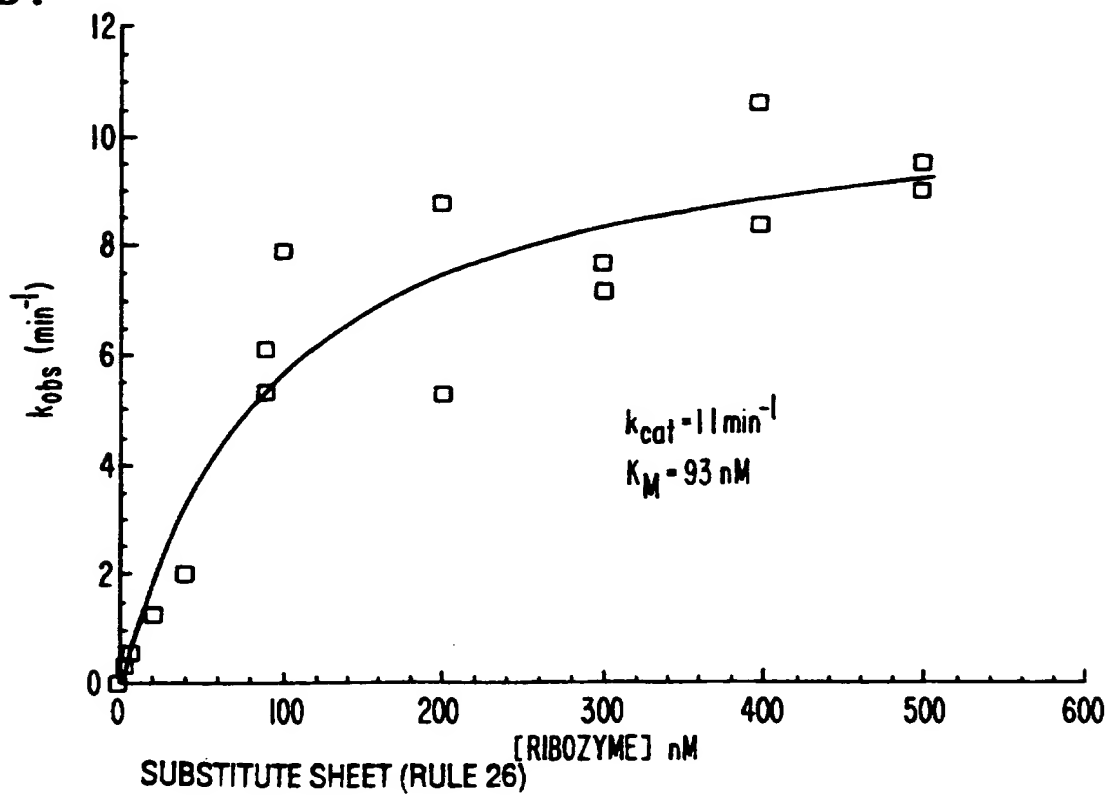


FIG. 44c.

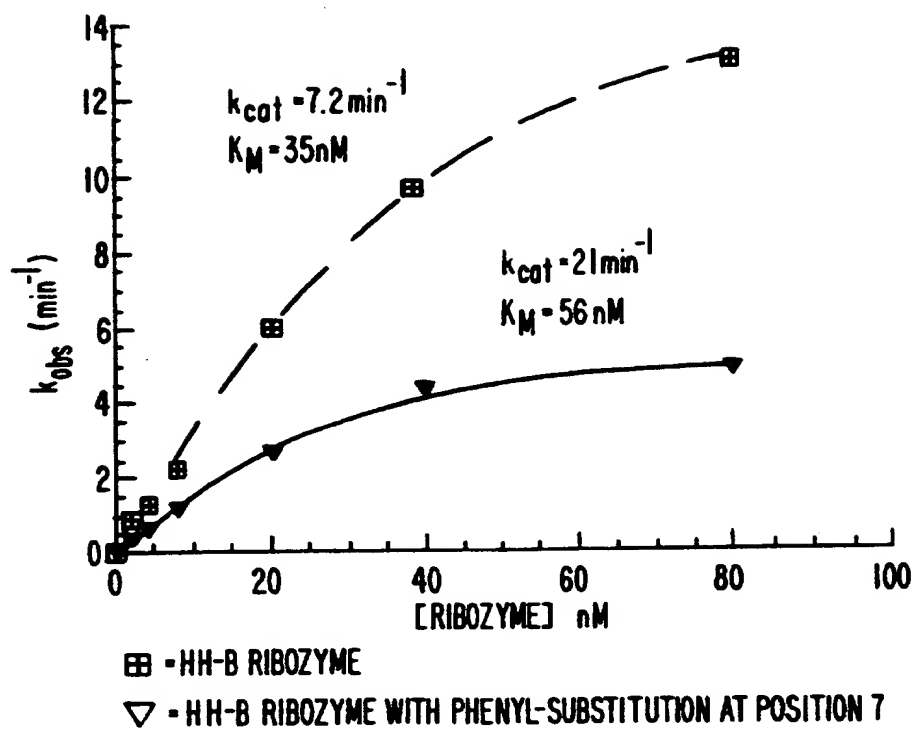
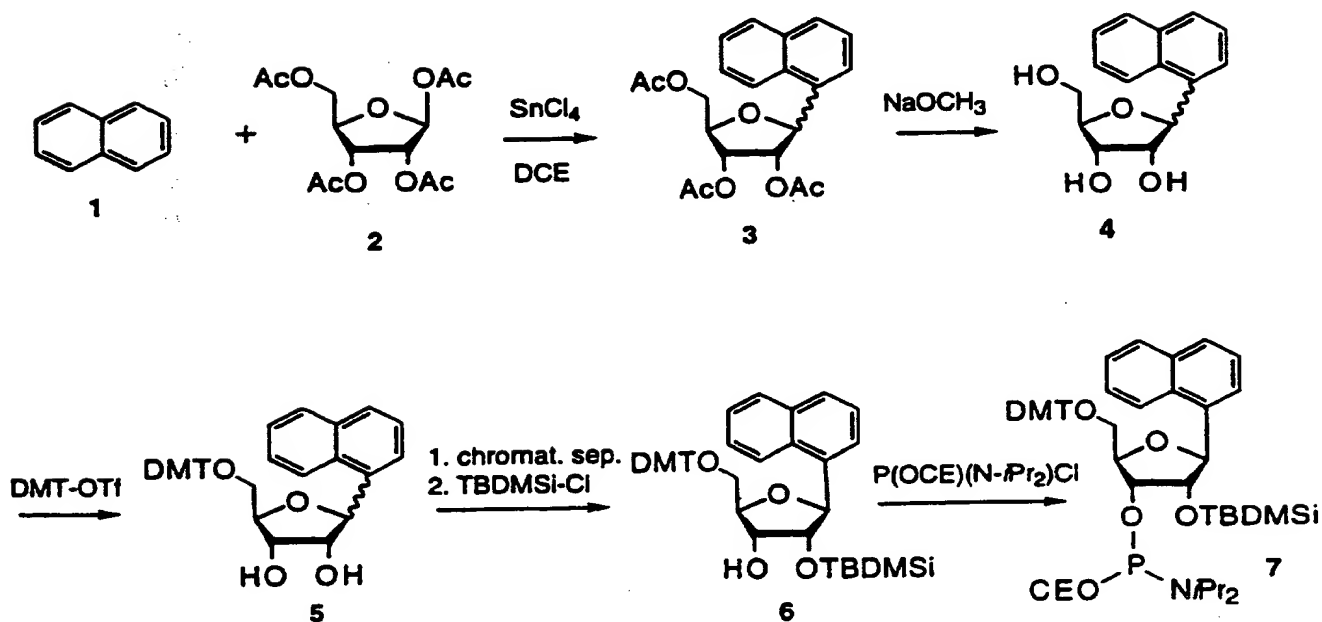
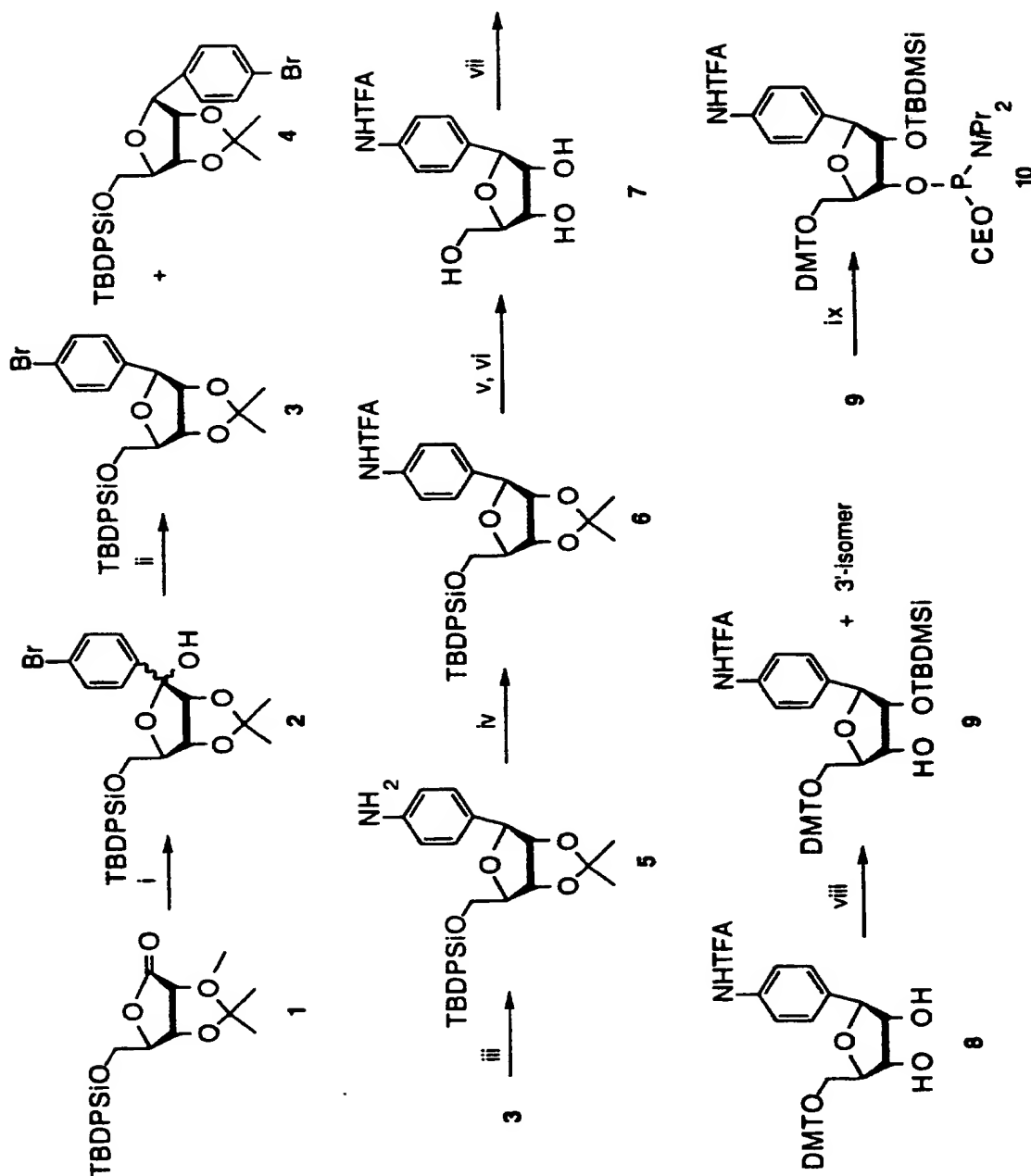


FIG. 45.

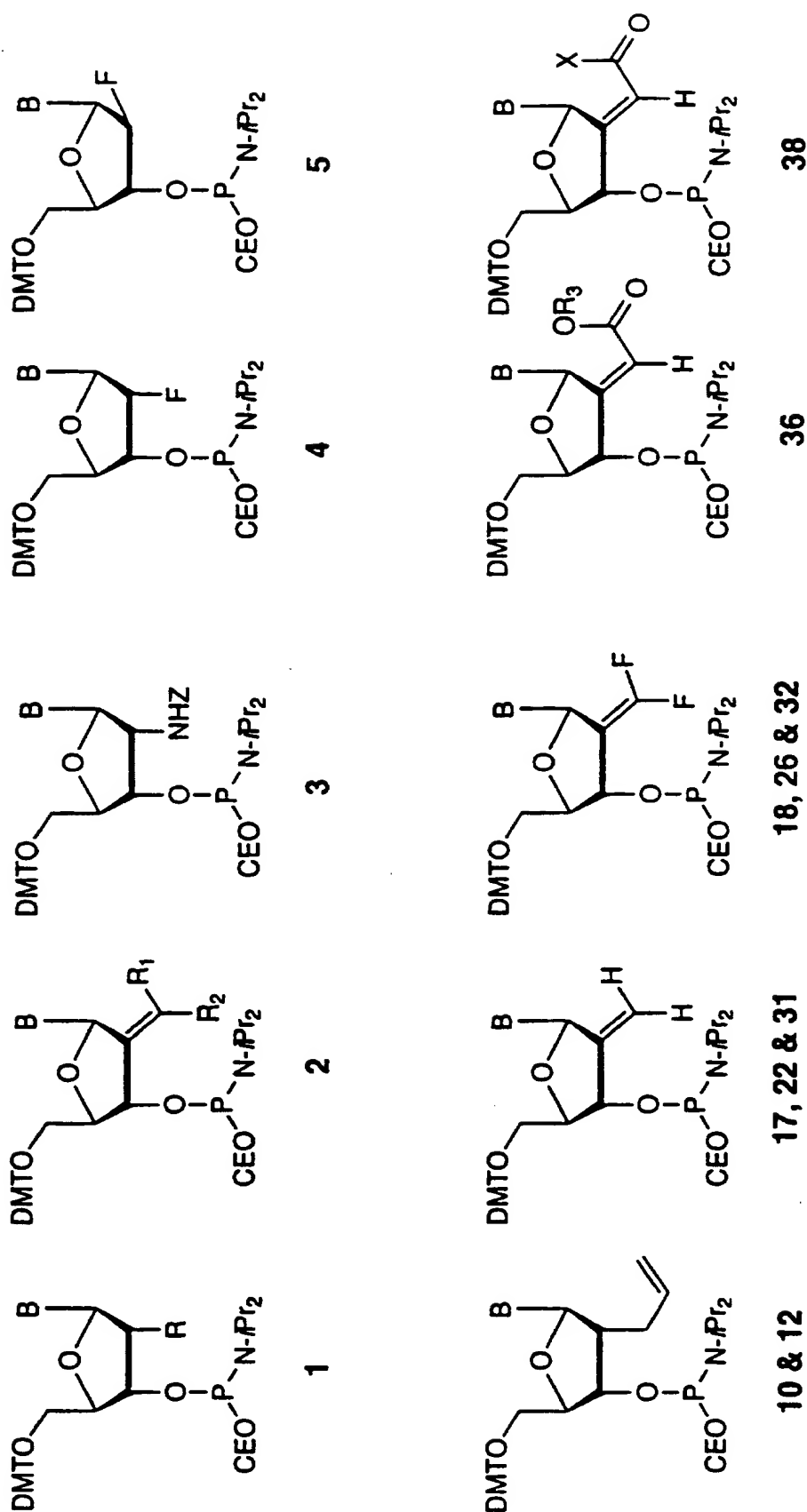


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FIG. 46.



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B = Protected A, C, G, U, T, 2AP, I, DiAP, P etc.

FIG. 48.

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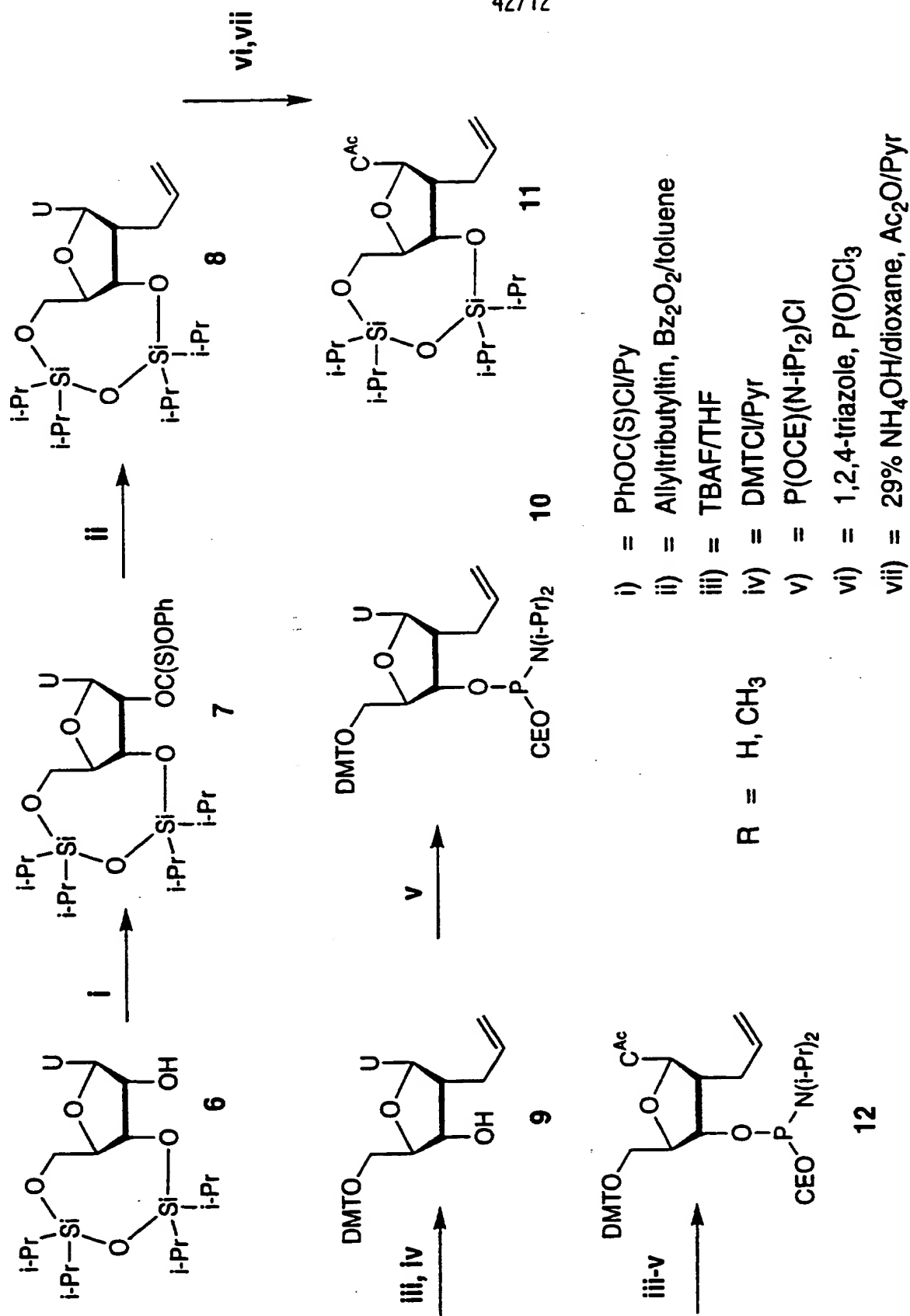


FIG. 49.

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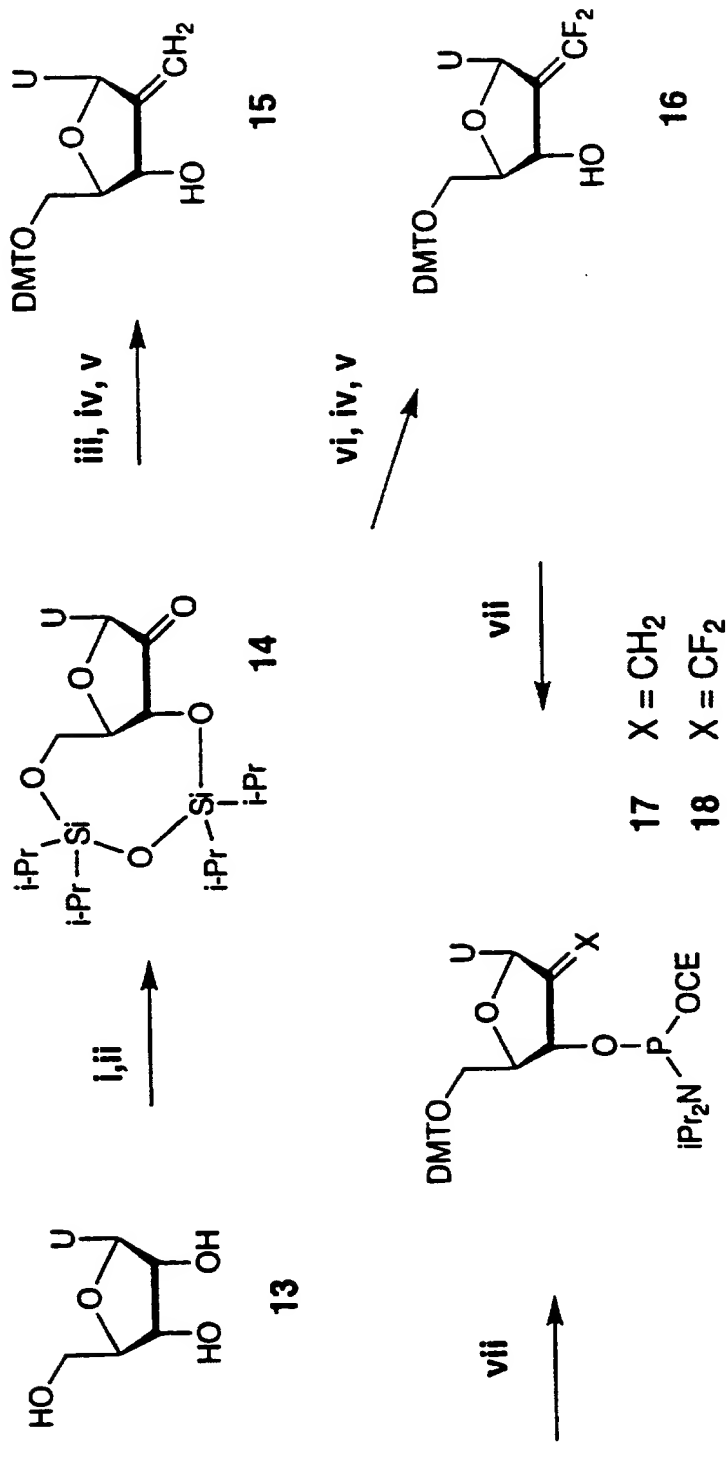


FIG. 50.

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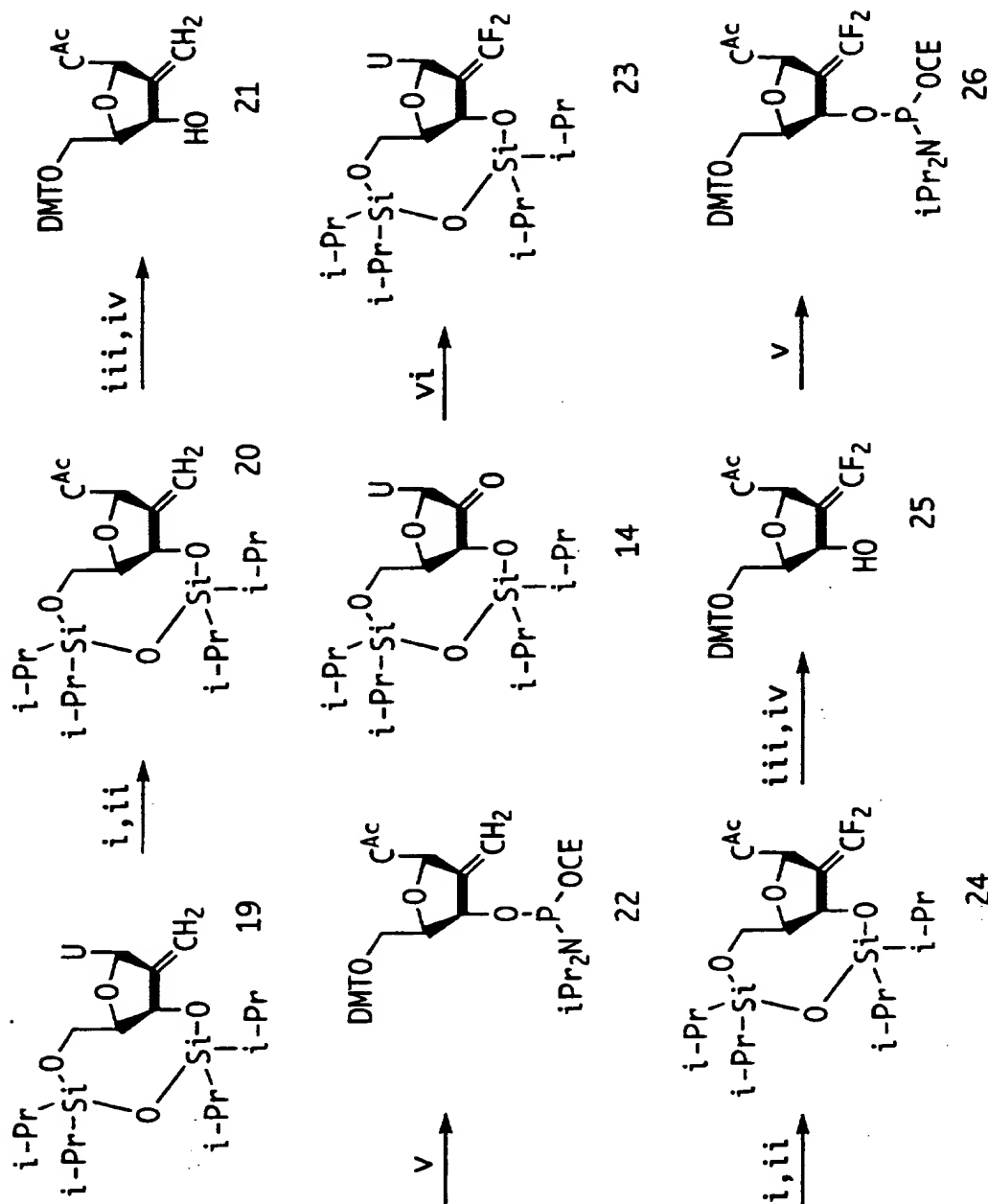
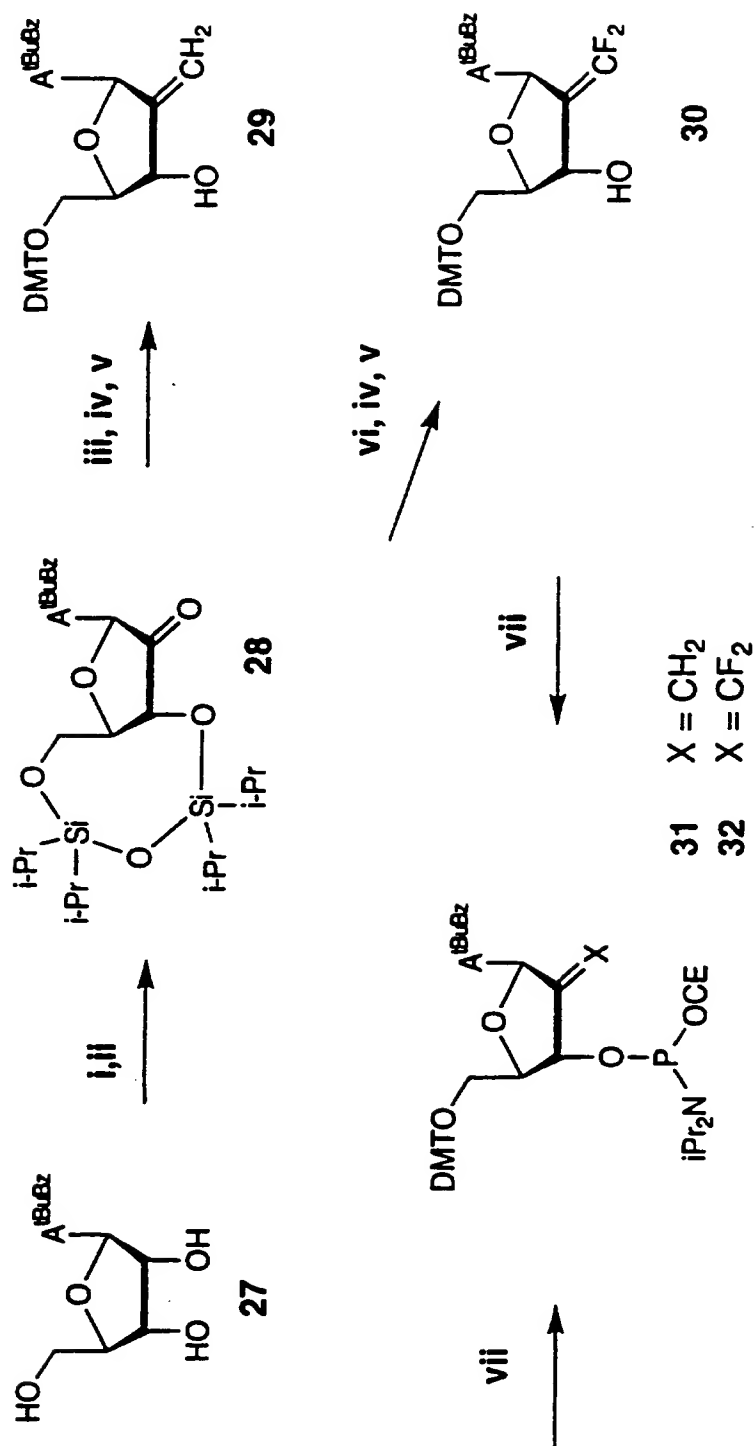


FIG. 51.

i) = 1,2,4-triazole, P(O)Cl₃ iii) = TBAF/THF v) = P(OCE)(N-iPr₂)Cl
 ii) = 29% NH₄OH/dioxane, Ac₂O/Pyr iv) = DMTCl/Pyr vi) = Ph₃P, ClCF₂COONa

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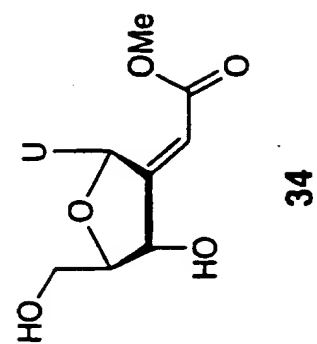


- i) = Markiewicz reagent
 ii) = DMSO & Ac₂O
 iii) = Ph₃PCH₃I
 iv) = TBAF/THF
 v) = DMTCI/Pyr
 vi) = Ph₃P, ClCF₂COONa
 vii) = P(OCE)(N-iPr₂)Cl

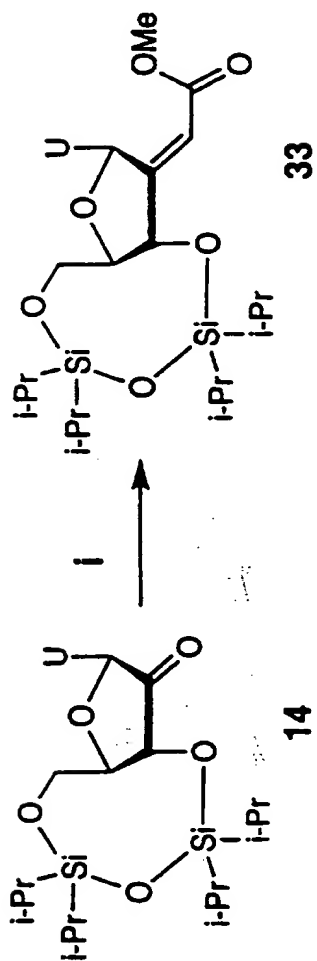
FIG. 52.

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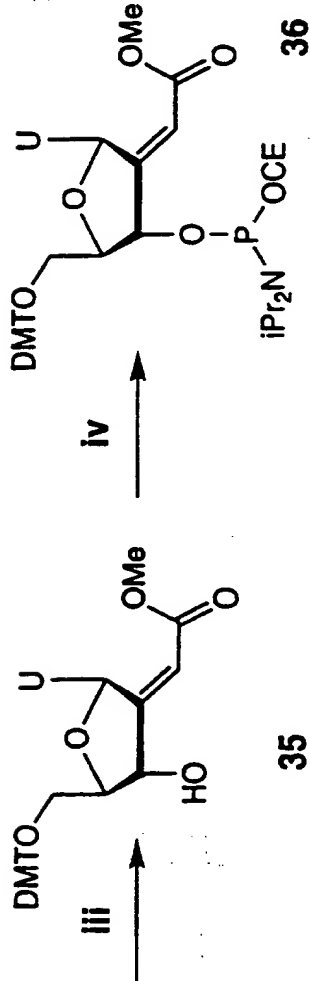
ii



i

33

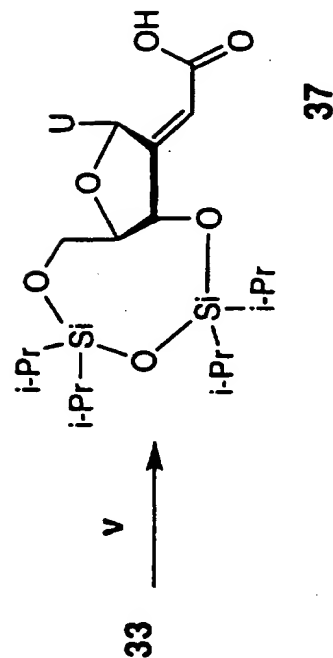
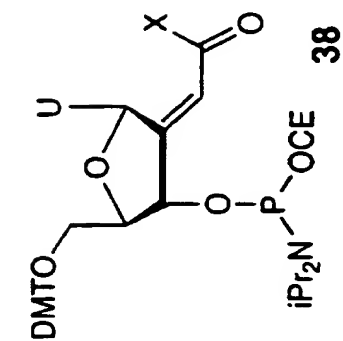
- i) = $\text{Ph}_3\text{PC}=\text{CHC}(\text{O})\text{OCH}_3\cdot\text{OAc}$
 ii) = $\text{NEt}_3\cdot 3 \text{ HF}$
 iii) = DMTCI/Pyr
 iv) = $\text{P}(\text{OCE})(\text{N-}i\text{Pr}_2)\text{Cl}$
 v) = MeOH/NaOH



iii

iv

36

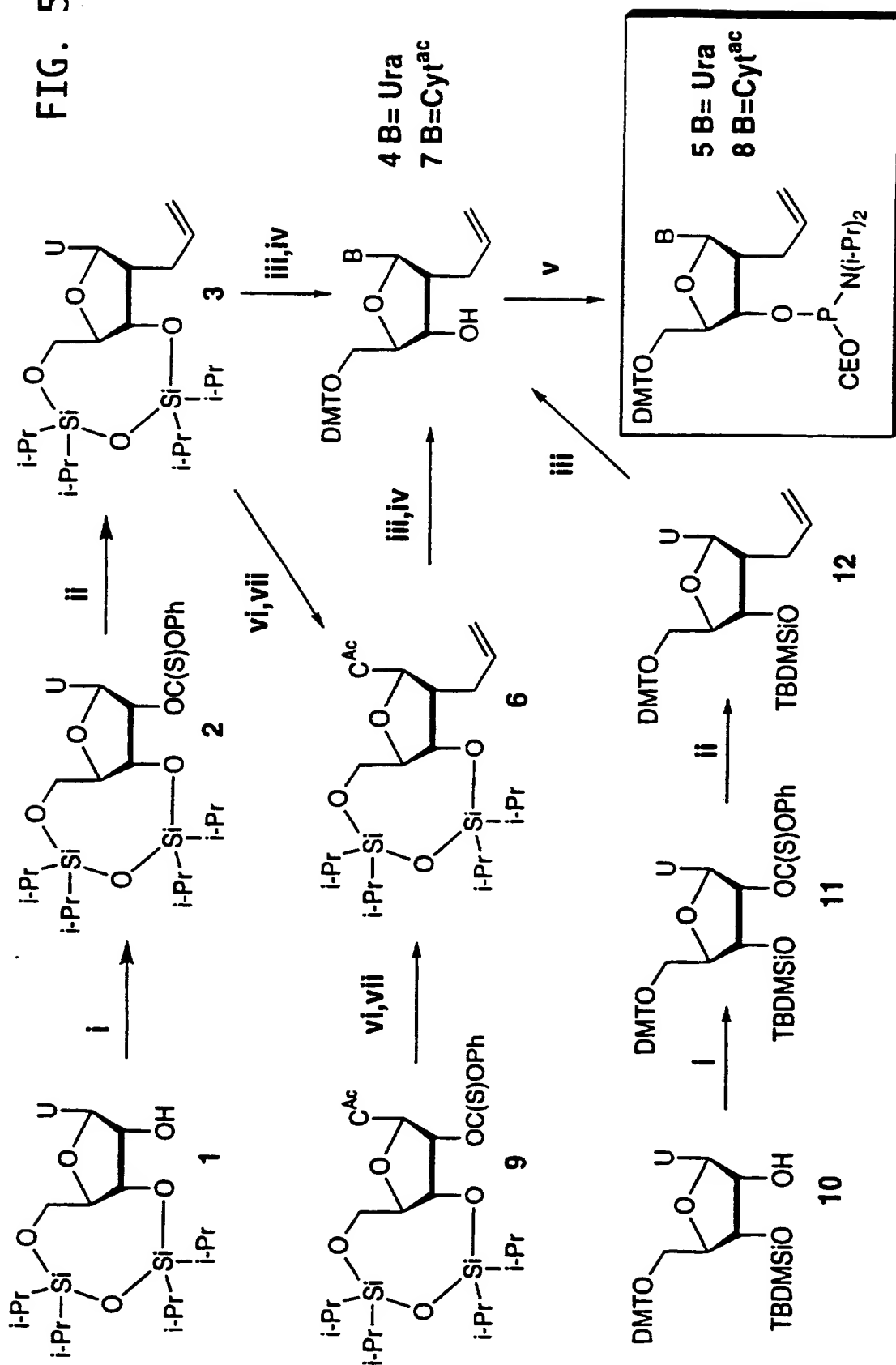


v

33

FIG. 53.

FIG. 54.



i) = PhOC(S)Cl/Py ii) = Allyltributyltin, Bz₂O₂ or AIBN /tolueneiii) = TBAF iv) = DMTCl/Py

$$v) = \text{P}(\text{OCE}(\text{N-IPr}_2)\text{Cl vi}) = 1,2,4\text{-triazole, P}(\text{O})\text{Cl}_3 \text{ vii}) = 29\% \text{ NH}_4\text{OH/dioxane, Ac}_2\text{O/Pyr}$$

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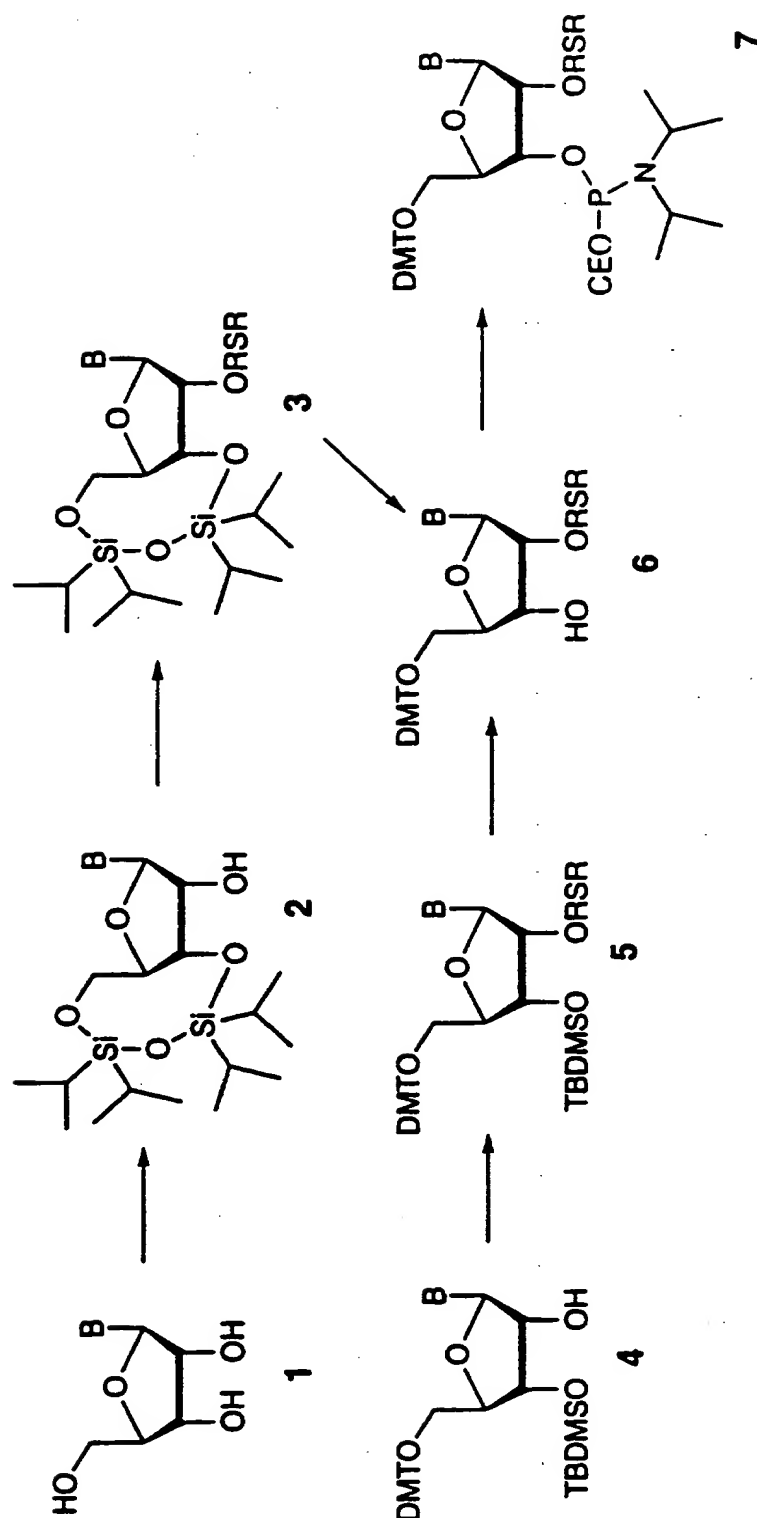


FIG. 55.

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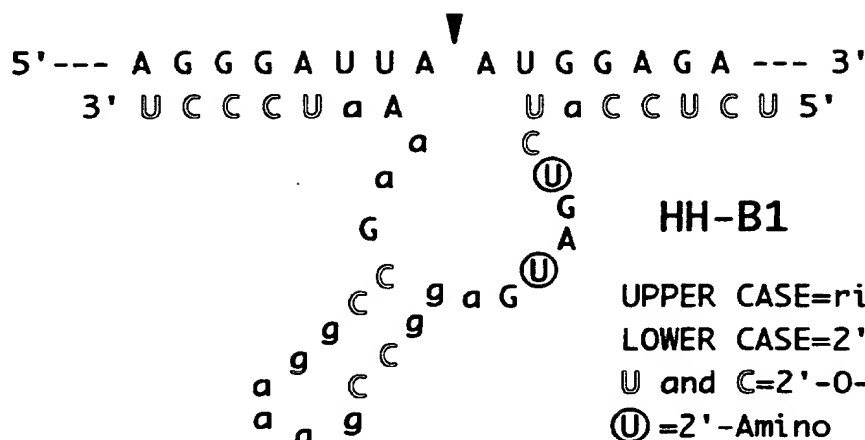


FIG. 56a.

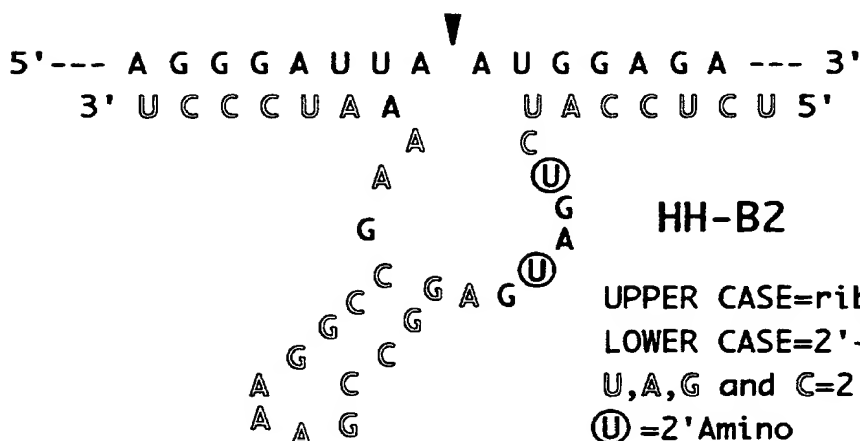


FIG. 56b.

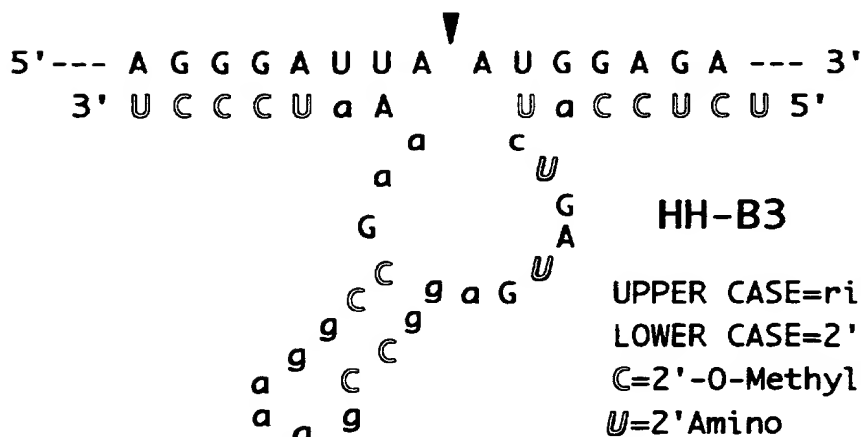


FIG. 56c.

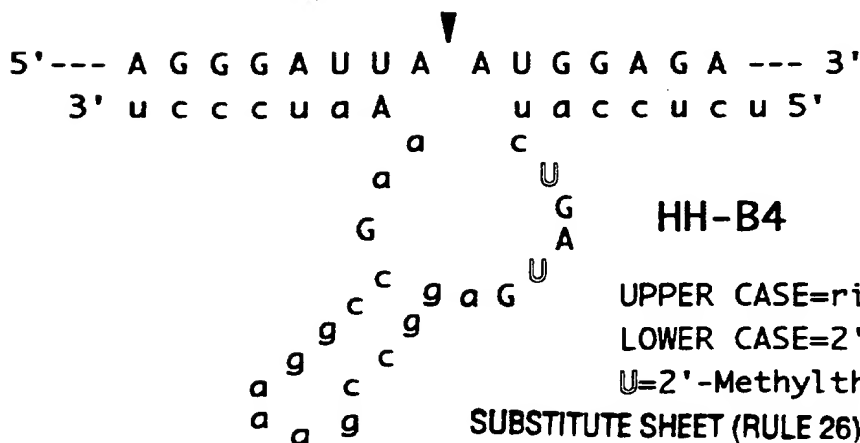


FIG. 56d.

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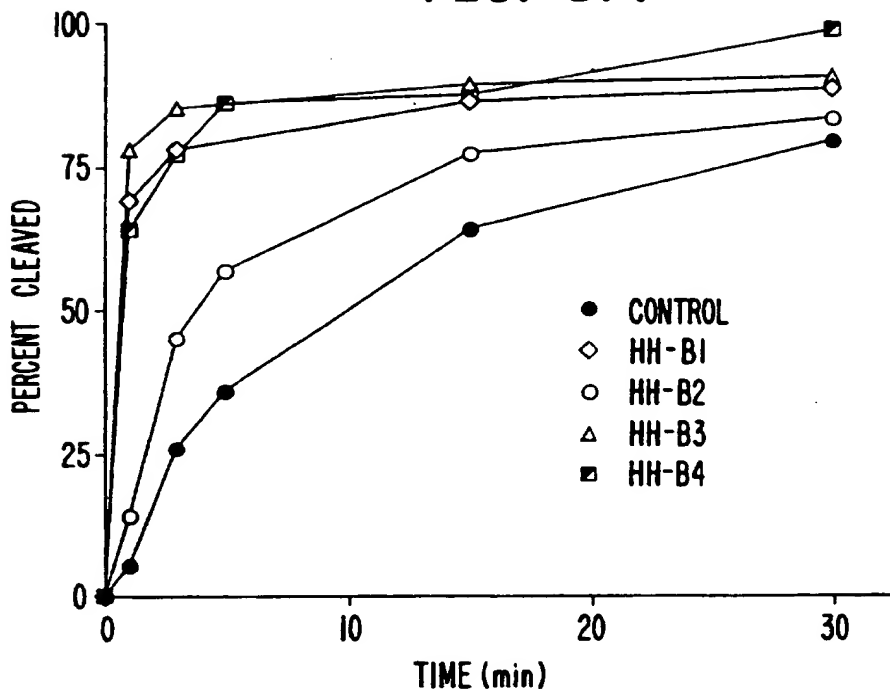
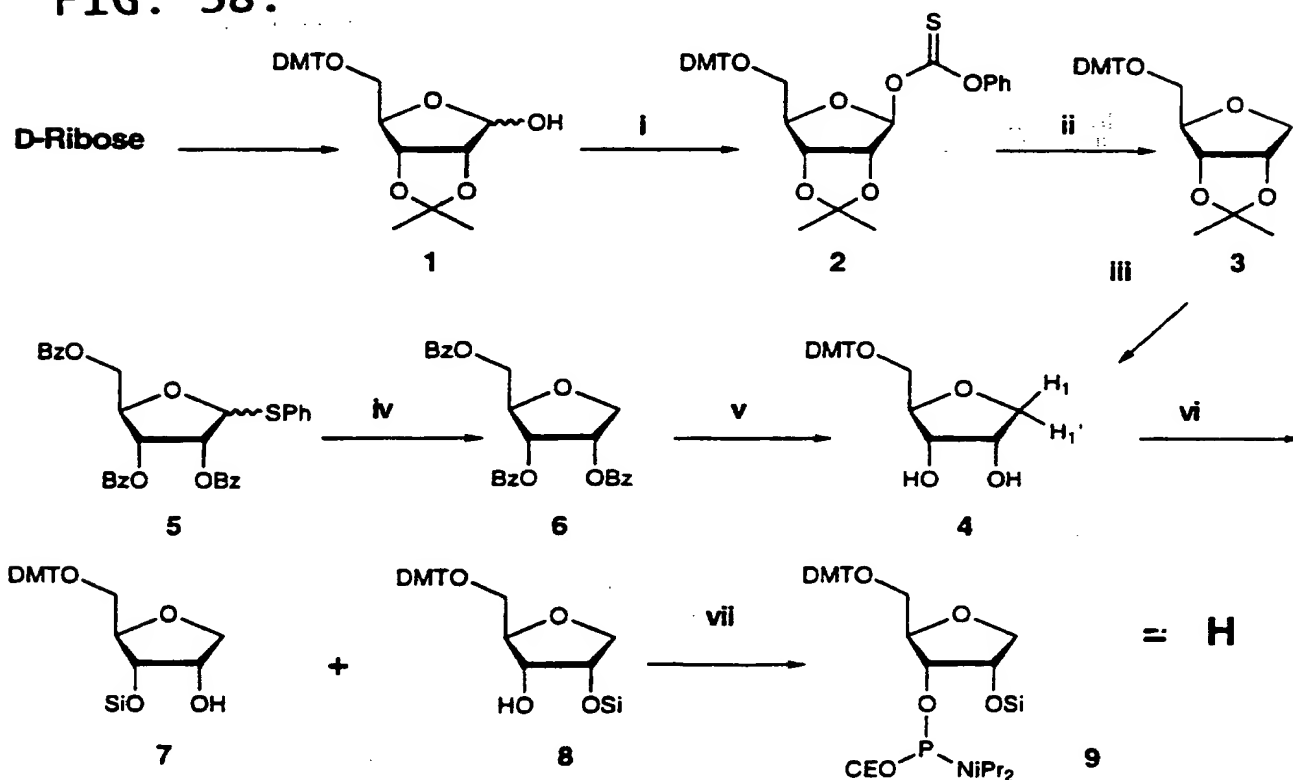
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FIG. 57.

FIG. 58.



Si = *t*-Butyldimethylsilyl
 DMT = 4,4'-Dimethoxytrityl
 CE = Cyanoethyl

Reagents and Conditions: i) PhOC(S)-Cl/DMAP, ii) Bu₃SnH/AIBN, iii) CF₃COOH, DMT-Cl/Pyr, iv) Bu₃SnH/Bz₂O₂, v) 2M NaOH/Pyr/MeOH, DMT-Cl/Pyr, vi) TBDMS-Cl/AgNO₃, vii) P(OCE)(N-*i*Pr₂)Cl
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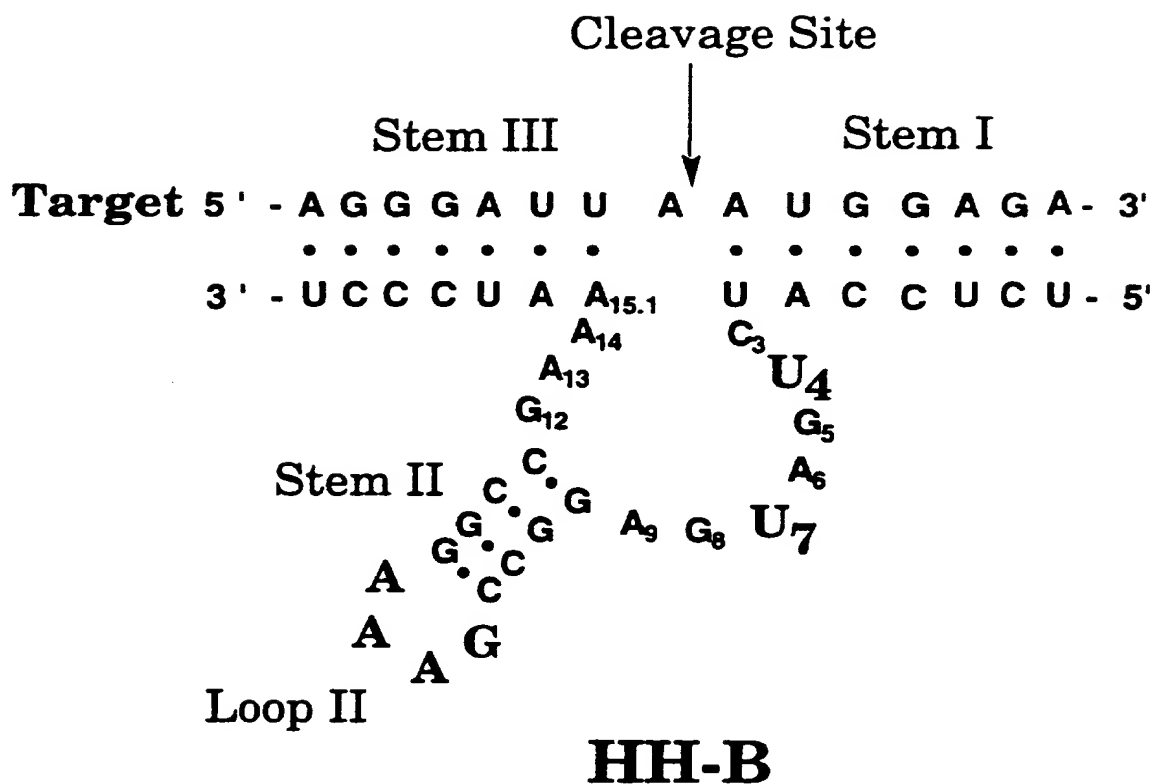


FIG. 59.

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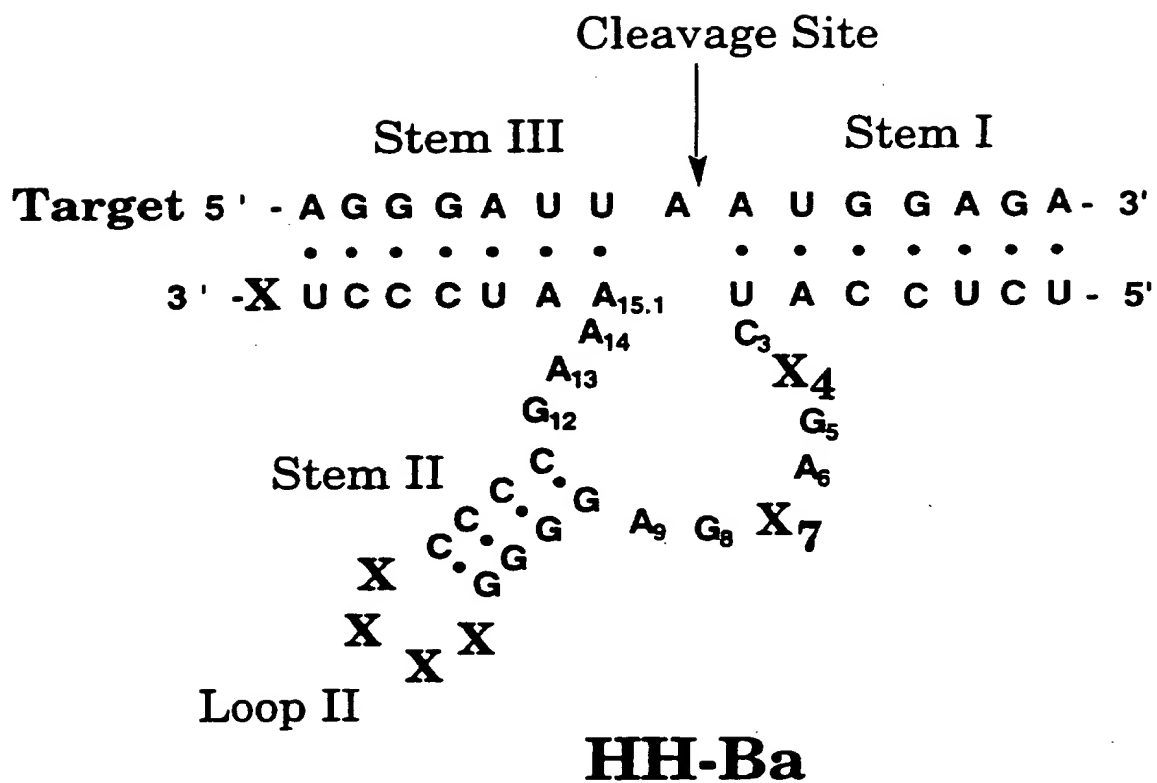


FIG. 60.

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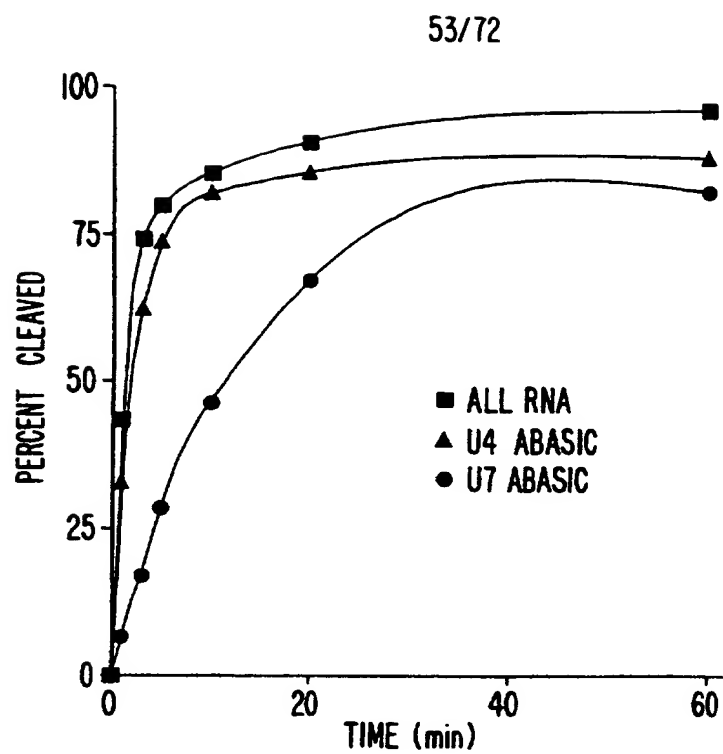


FIG. 61.

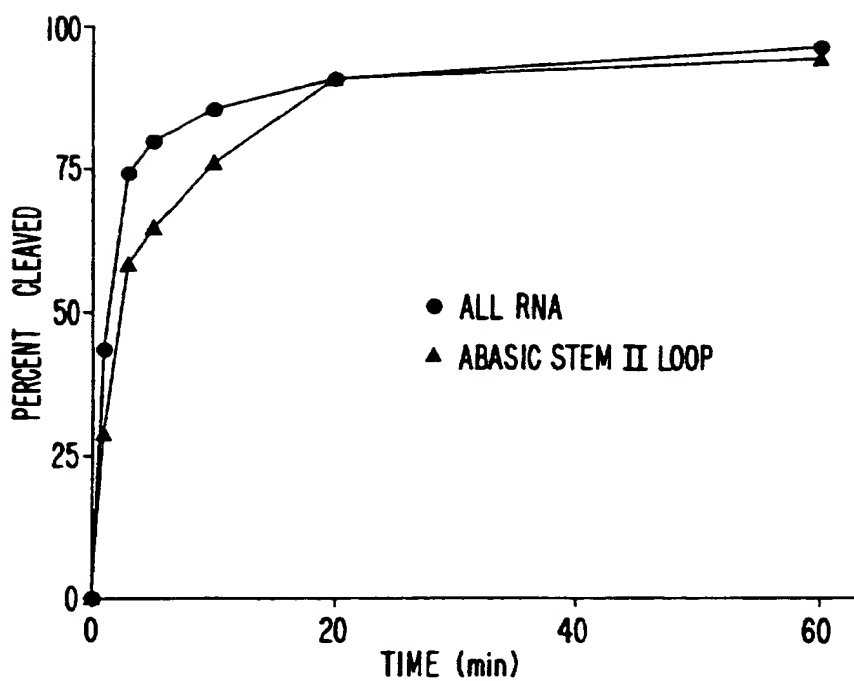


FIG. 62.

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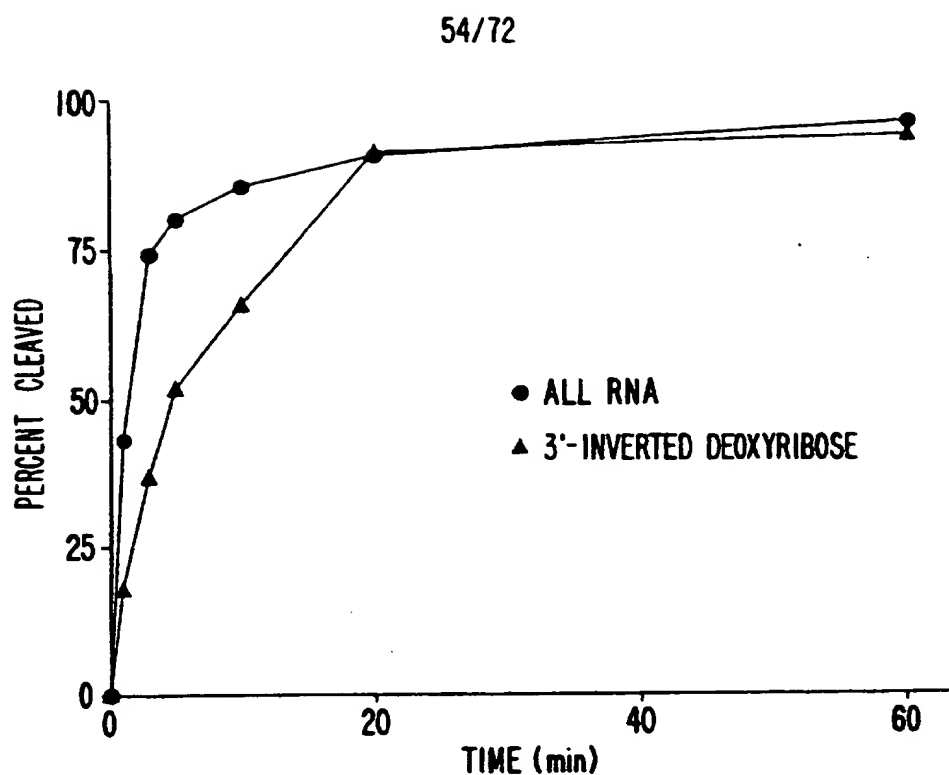


FIG. 63.

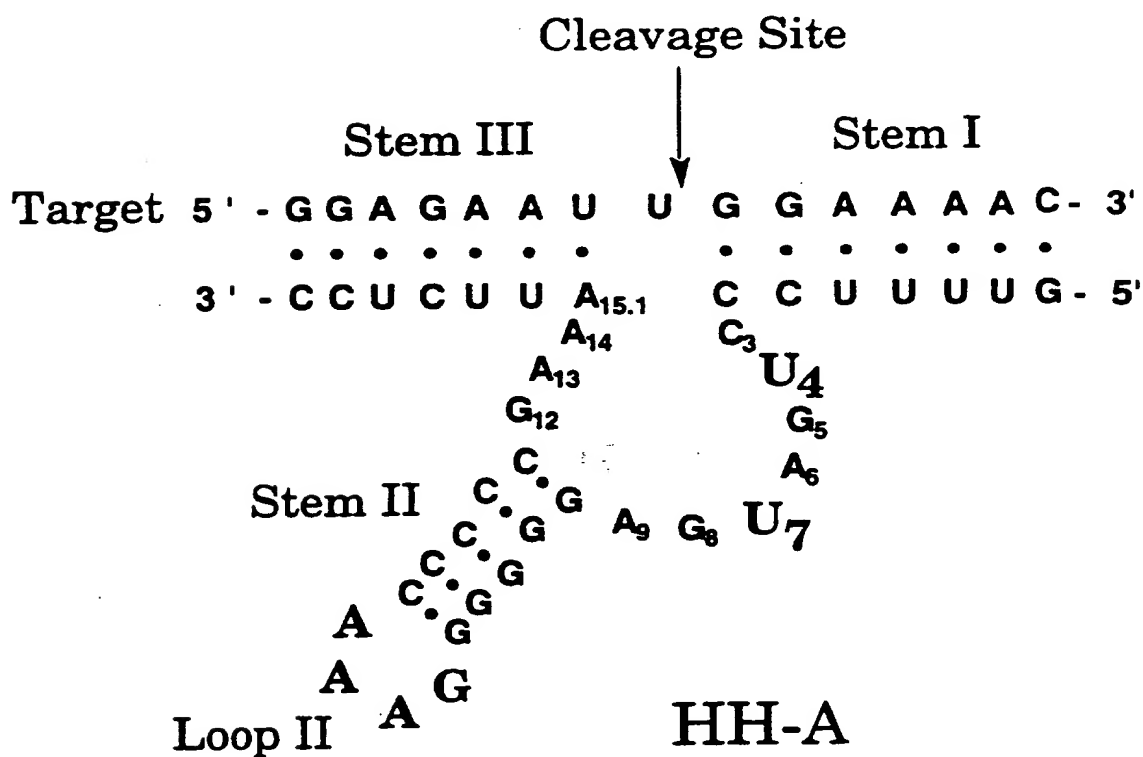


FIG. 64.

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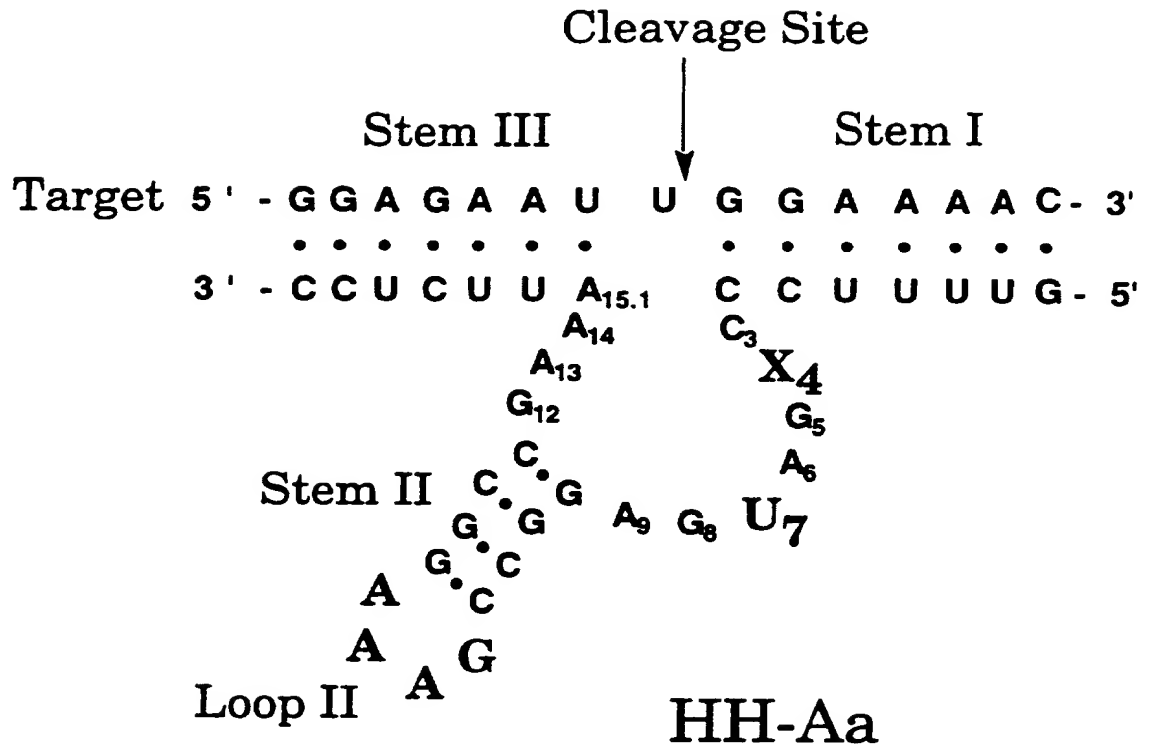


FIG. 65.

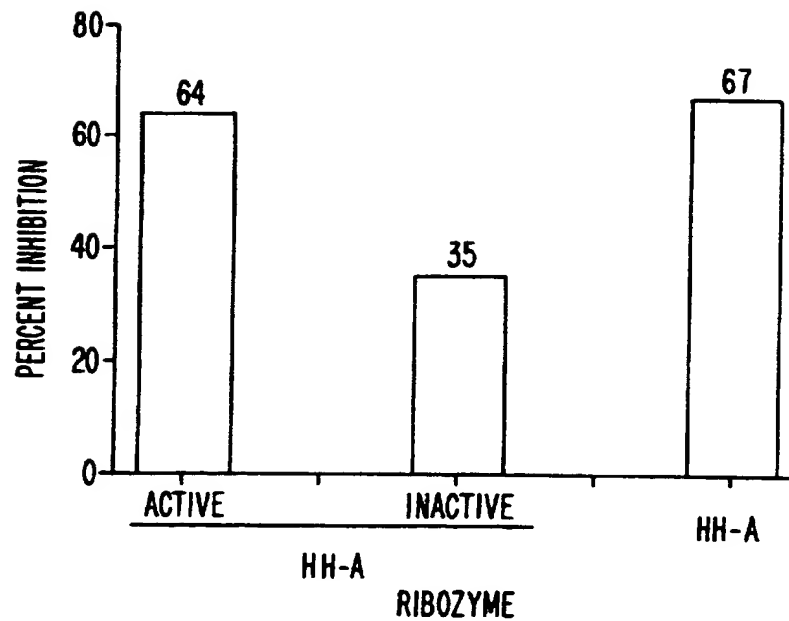


FIG. 66.

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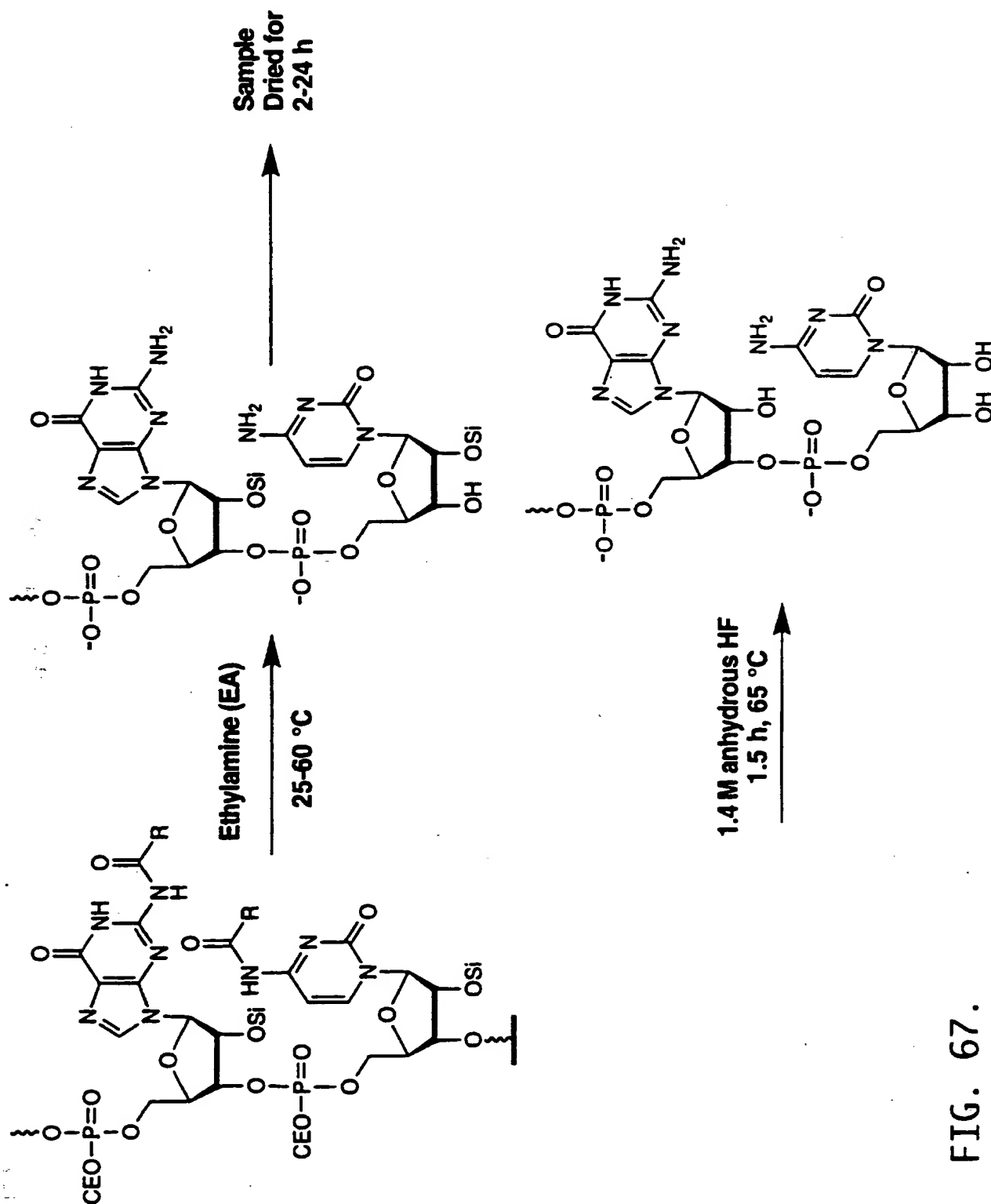


FIG. 67.

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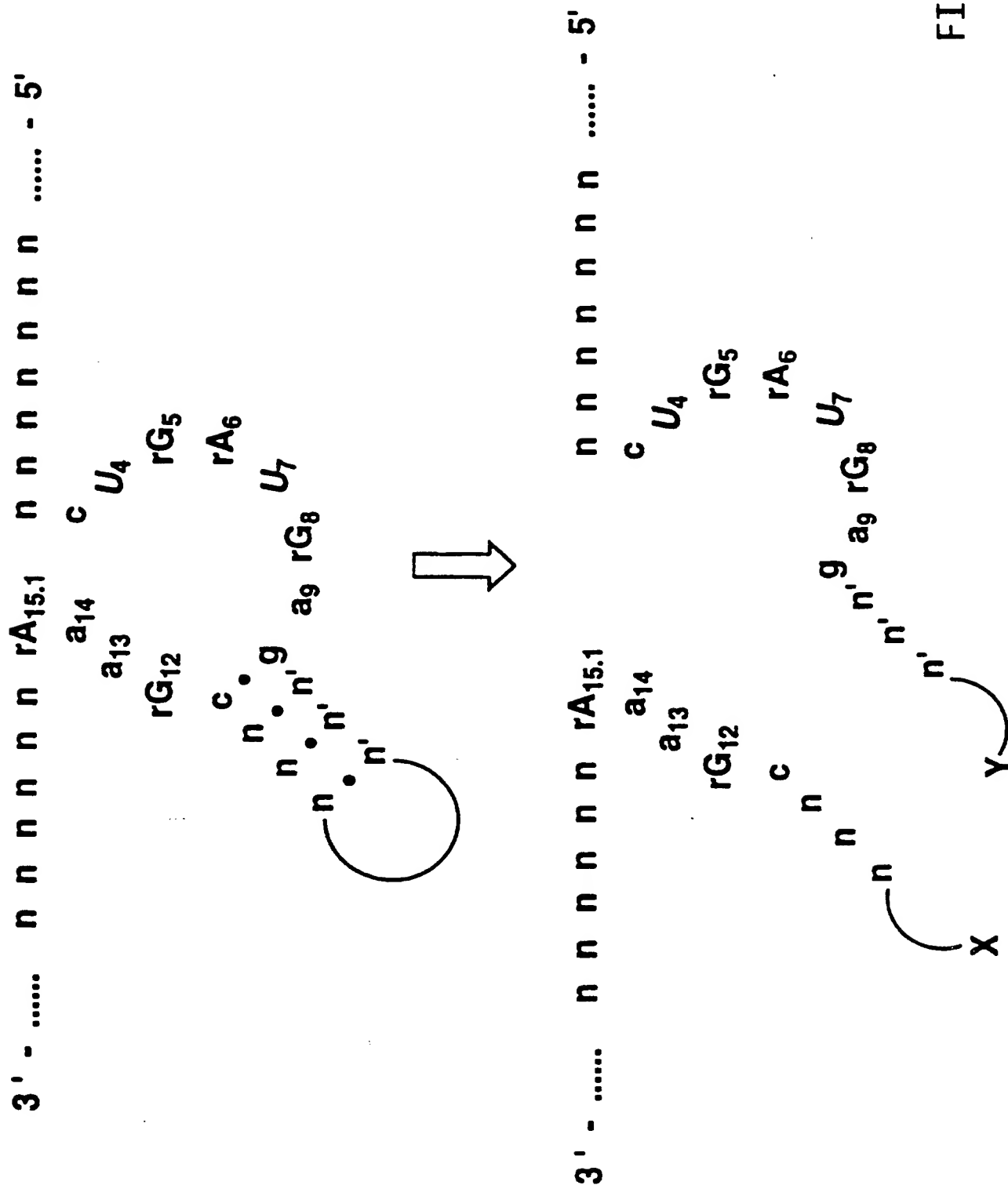
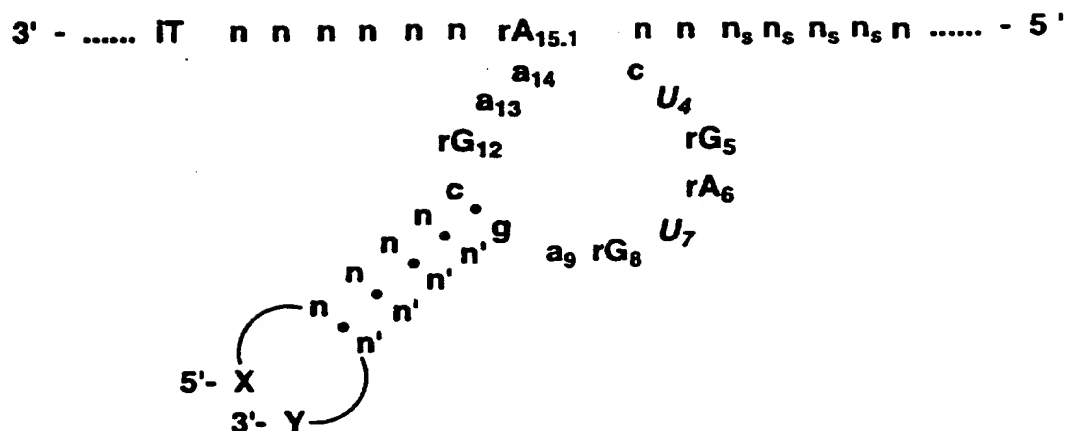


FIG. 68.

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NOTE: $(CH_2)_n$ refers to any linkage. In addition, X and Y can be interchanged.

$$X = (\text{CH}_2)_n\text{SH}, Y = (\text{CH}_2)_n\text{SH} \rightarrow \text{disulfide}$$

X = (CH₂)_nNHR, Y = ribose → morpholino

$X = (CH_2)_nNHR$, $Y = CO_2H \longrightarrow$ amide

$$X = (\text{CH}_2)_n\text{X}, \quad Y = (\text{CH}_2)_n\text{OH} \rightarrow \text{ether, X = halogen}$$
$$X = (\text{CH}_2)_n\text{NHR}, Y = \text{CHO} \longrightarrow \text{amine}$$

X = (CH₂)_nPPh₃, Y = CHO → double bond

X = (CH₂)_nNHR, Y = (CH₂)_nSO₂Cl → sulfonamide

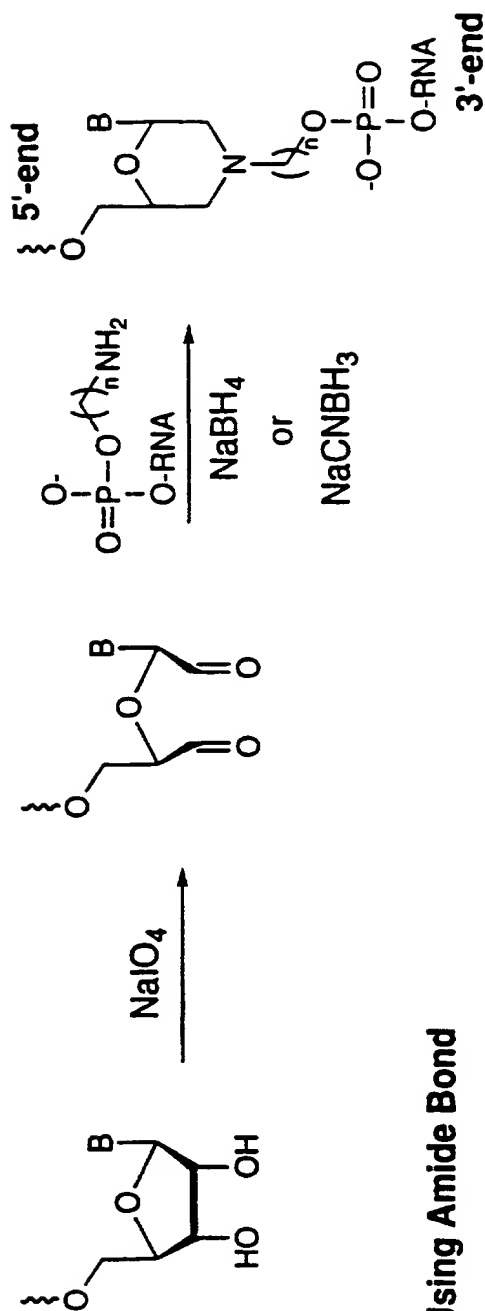
$$X = (\text{CH}_2)_n\text{OH}, Y = \text{CO}_2\text{H} \quad \longrightarrow \quad \text{ester}$$
$$\text{X} = (\text{CH}_2)_n\text{X}, \quad \text{Y} = (\text{CH}_2)_n\text{SH} \longrightarrow \text{thioether, X = halogen}$$
$$X = (\text{CH}_2)_n\text{COX}, \quad Y = (\text{CH}_2)_n\text{OH} \rightarrow \text{carbonate, } X = \text{halogen}$$

FIG. 69.

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Using Morpholino Group



Using Amide Bond

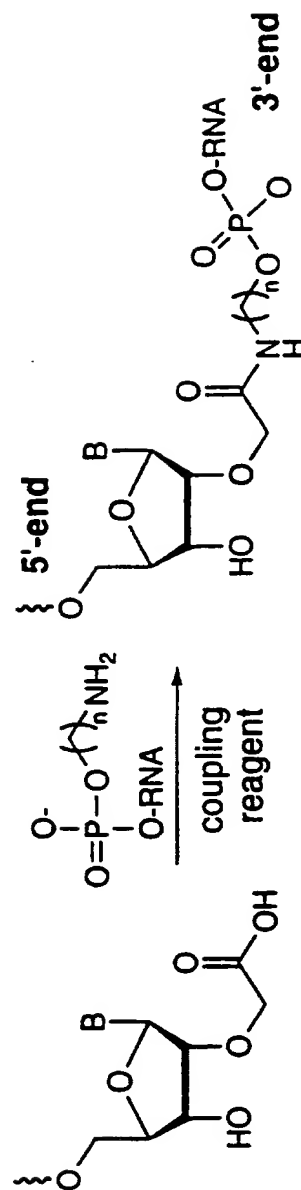


FIG. 70.

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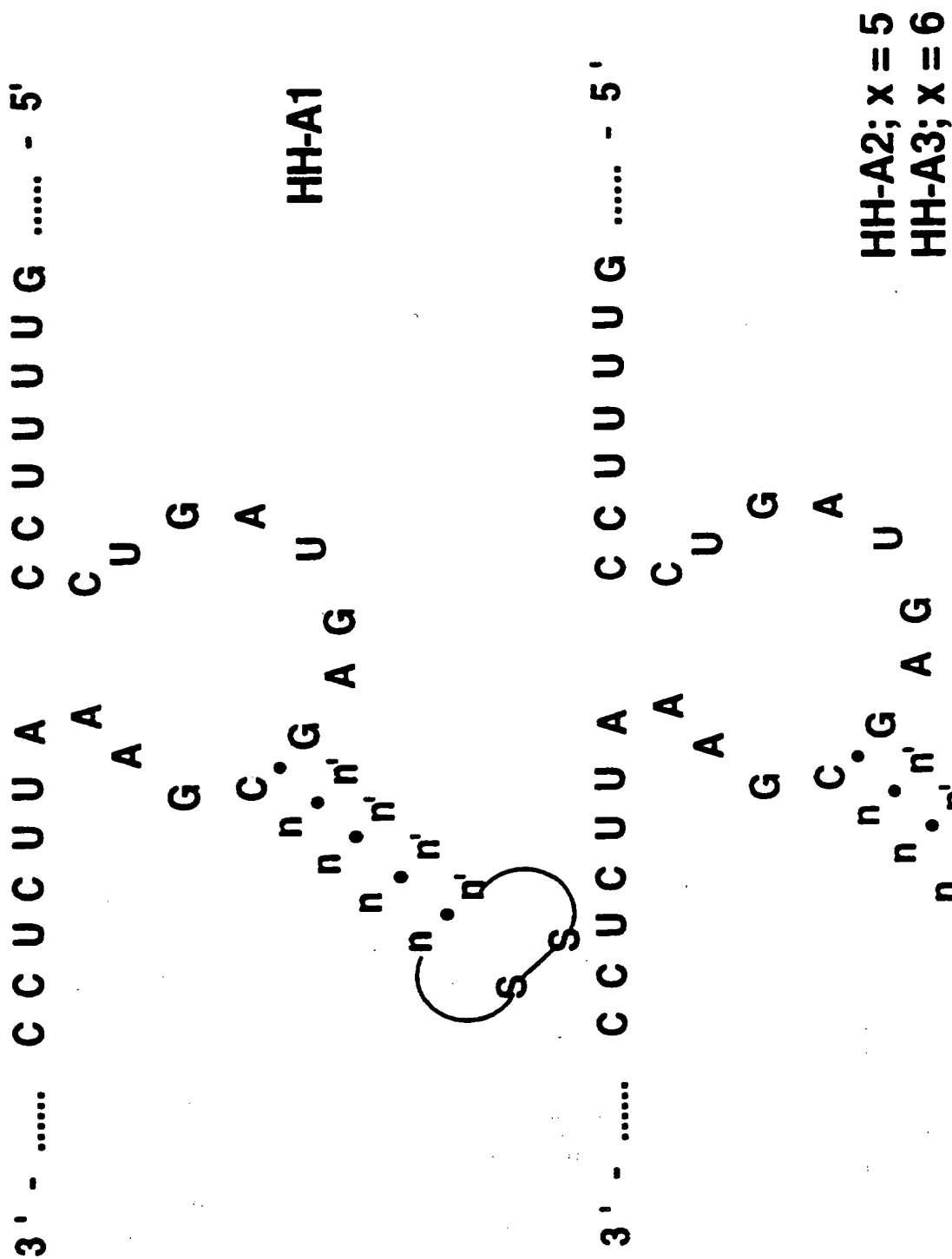


FIG. 71.

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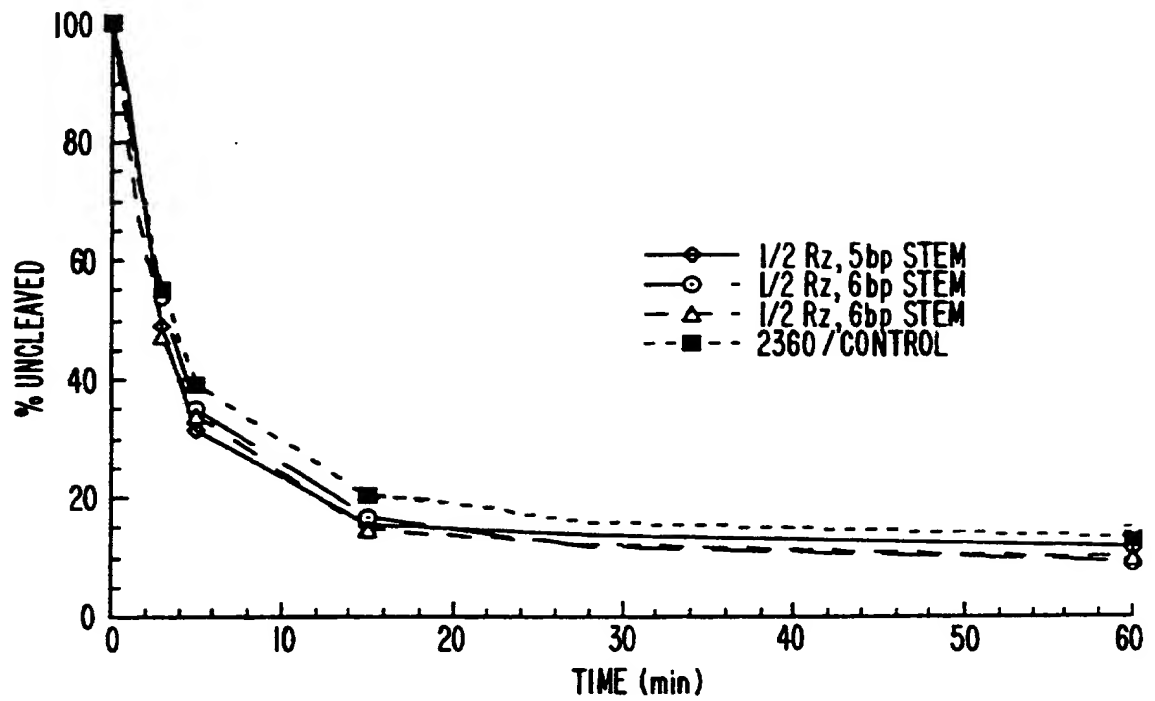


FIG. 72.

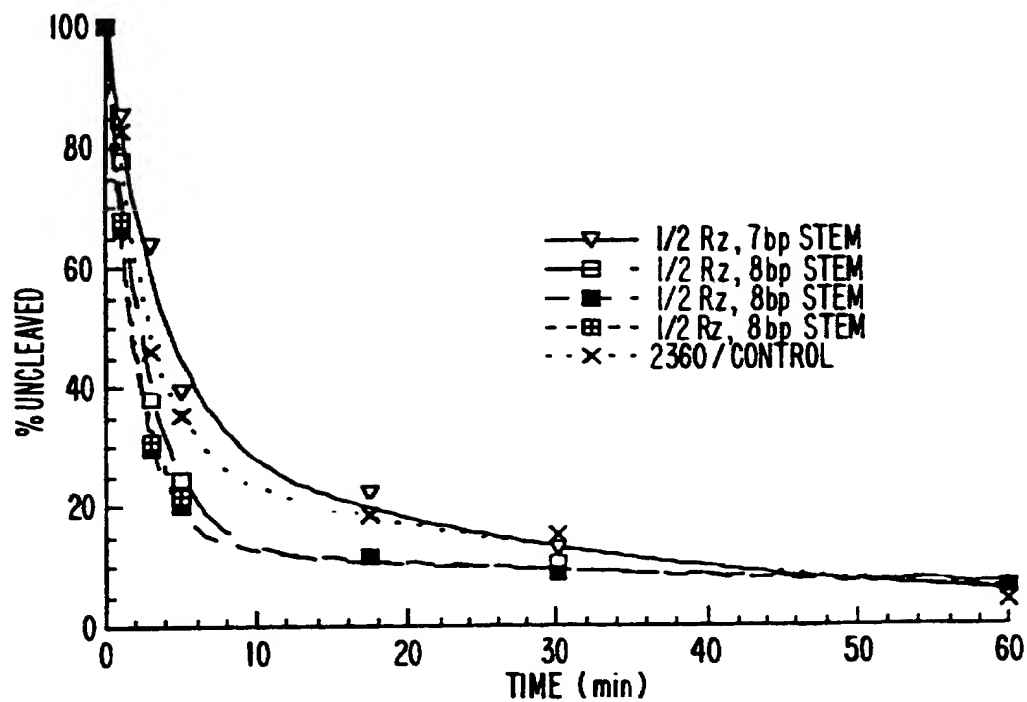


FIG. 73.

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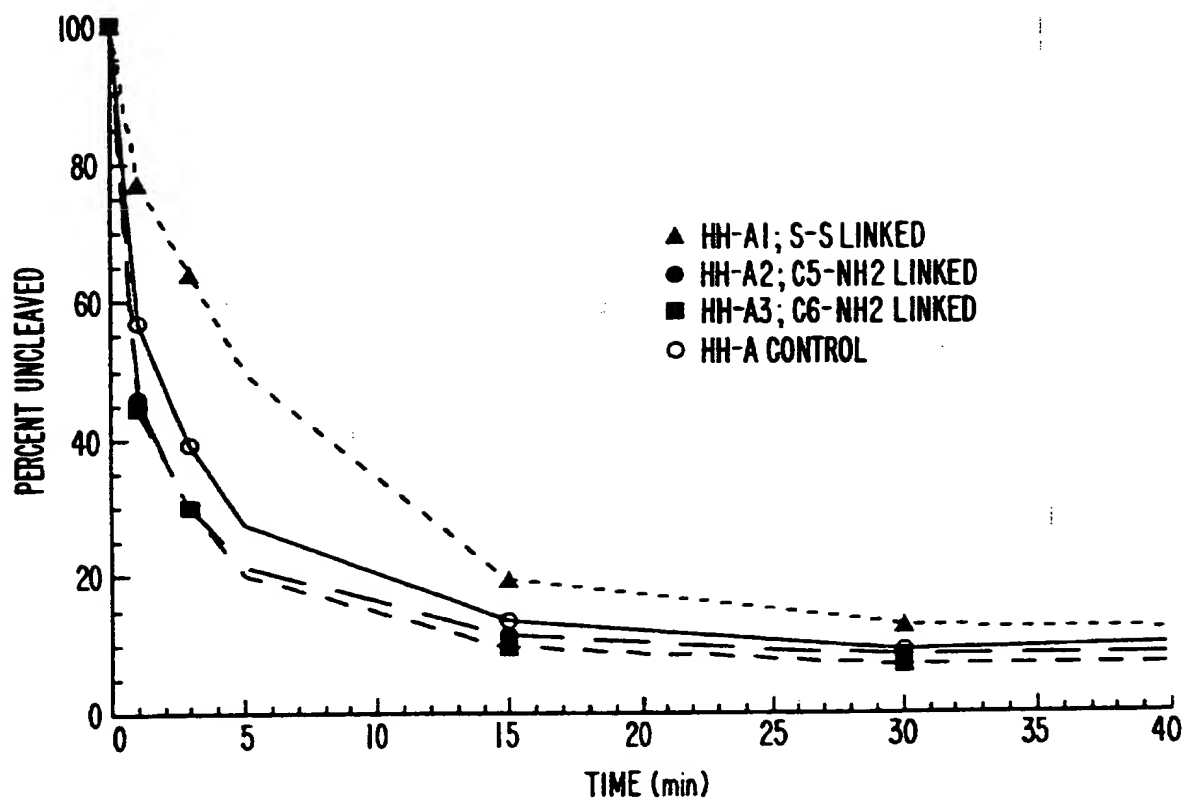
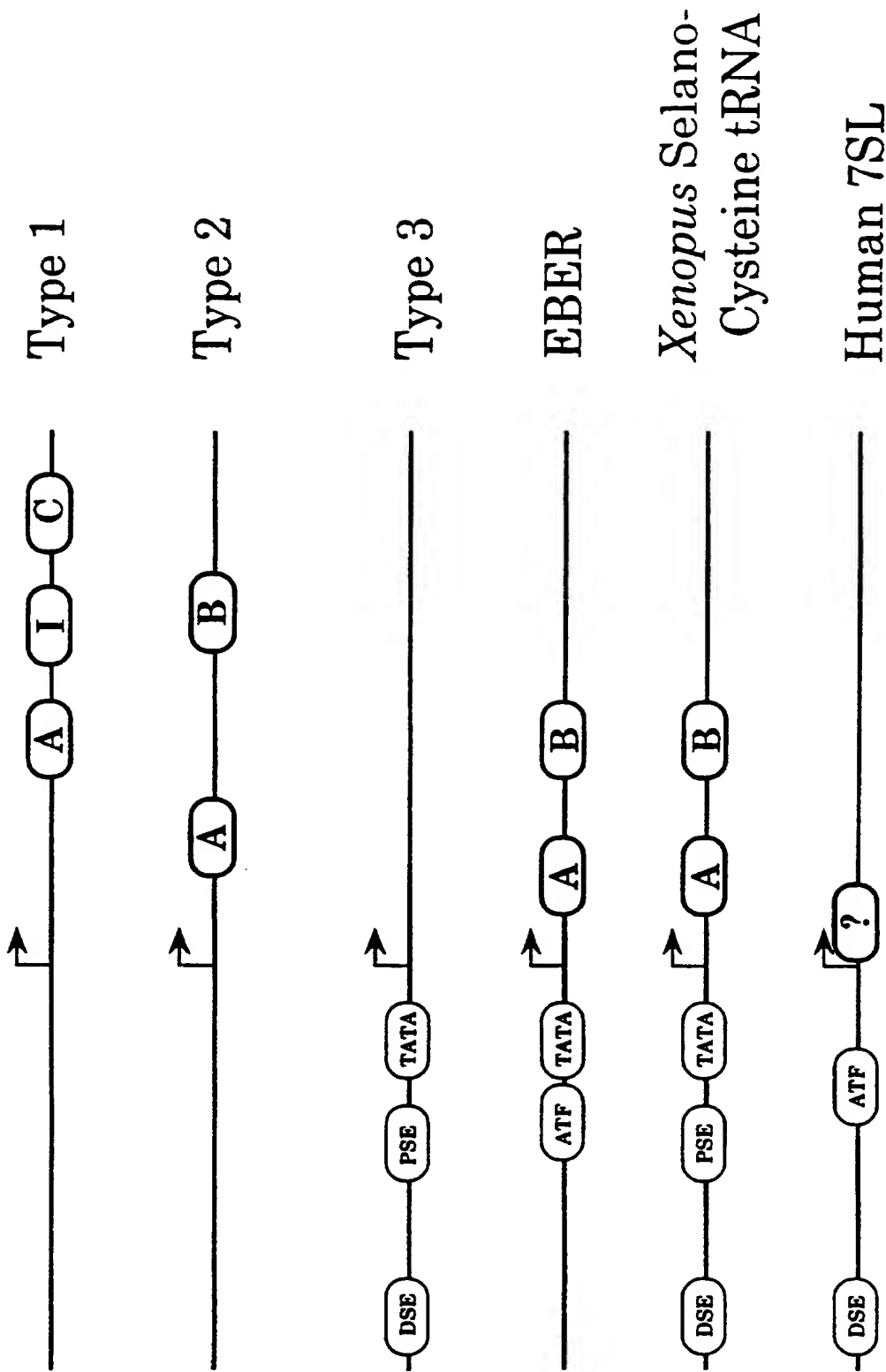
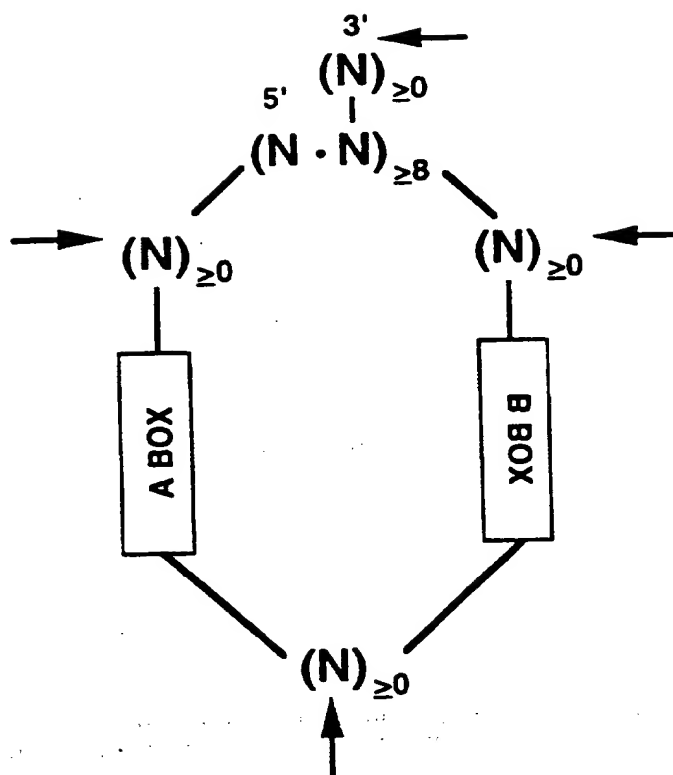


FIG. 74.

FIG. 75.



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A BOX = URGCNNAGYGG

B BOX = GGUUCGANUCC

This is based on Geiduschek & Tocchini-Valentini, (1988) Annu. Review Biochem. 57, 873-914. However this consensus sequence is not meant to be limiting

N = A, U, G, or C

R = Purine

Y = Pyrimidine

• = Indicates base-pairing

— = Indicates covalent linkage

➔ = Indicates sites at which desired RNAs can be cloned

FIG. 76.

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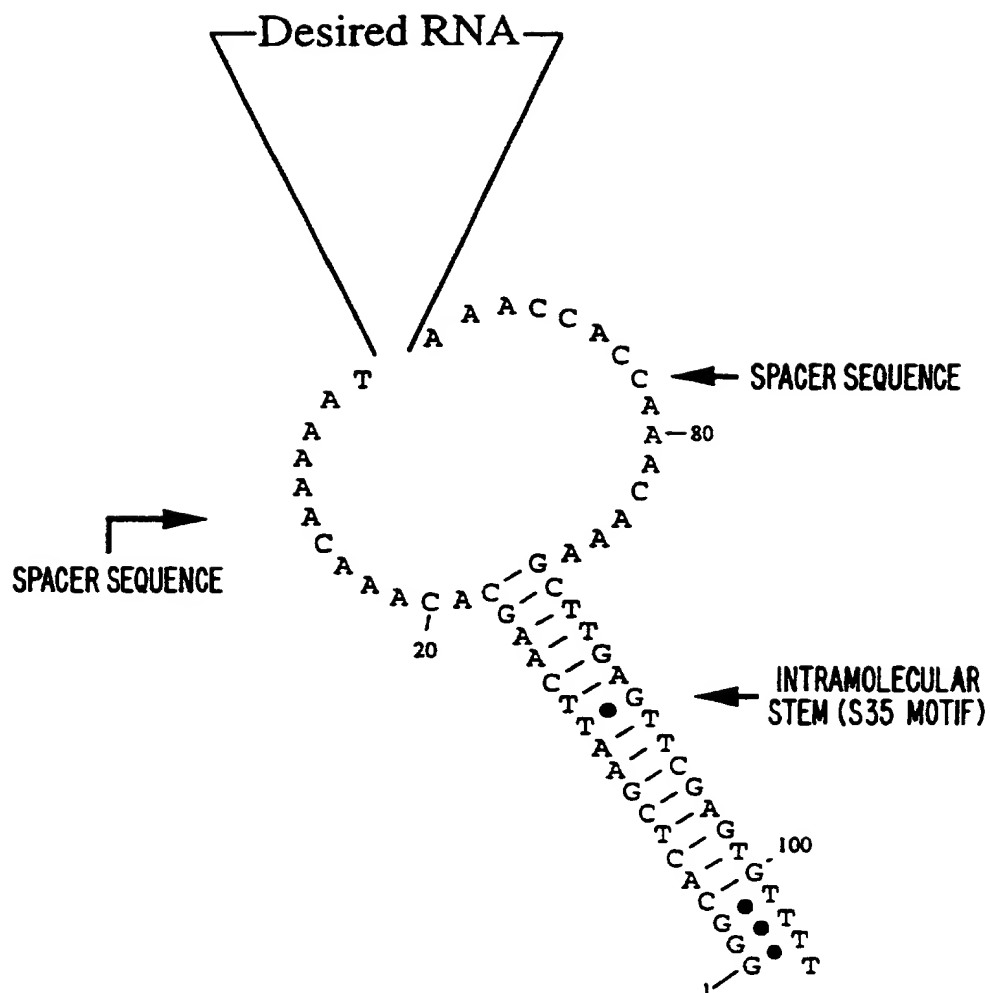
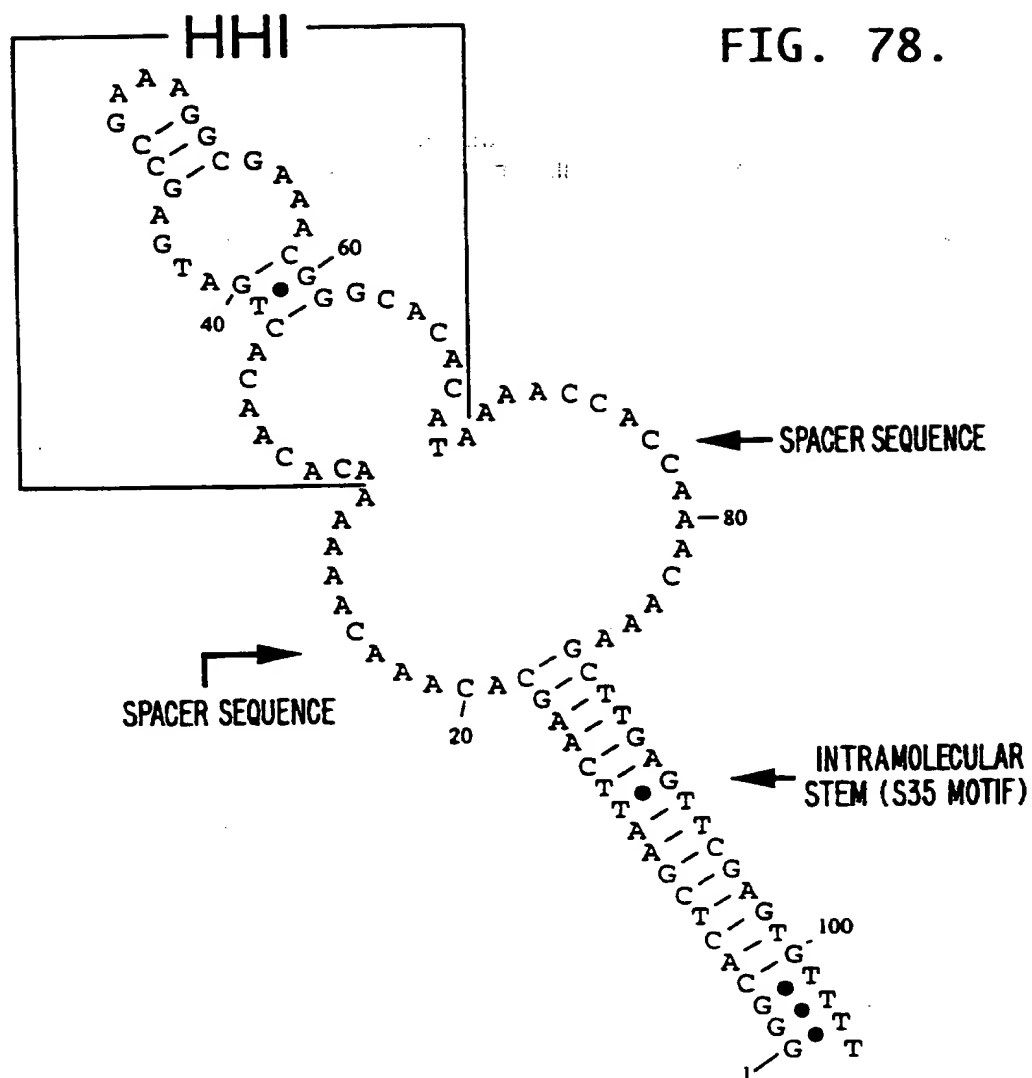


FIG. 77.

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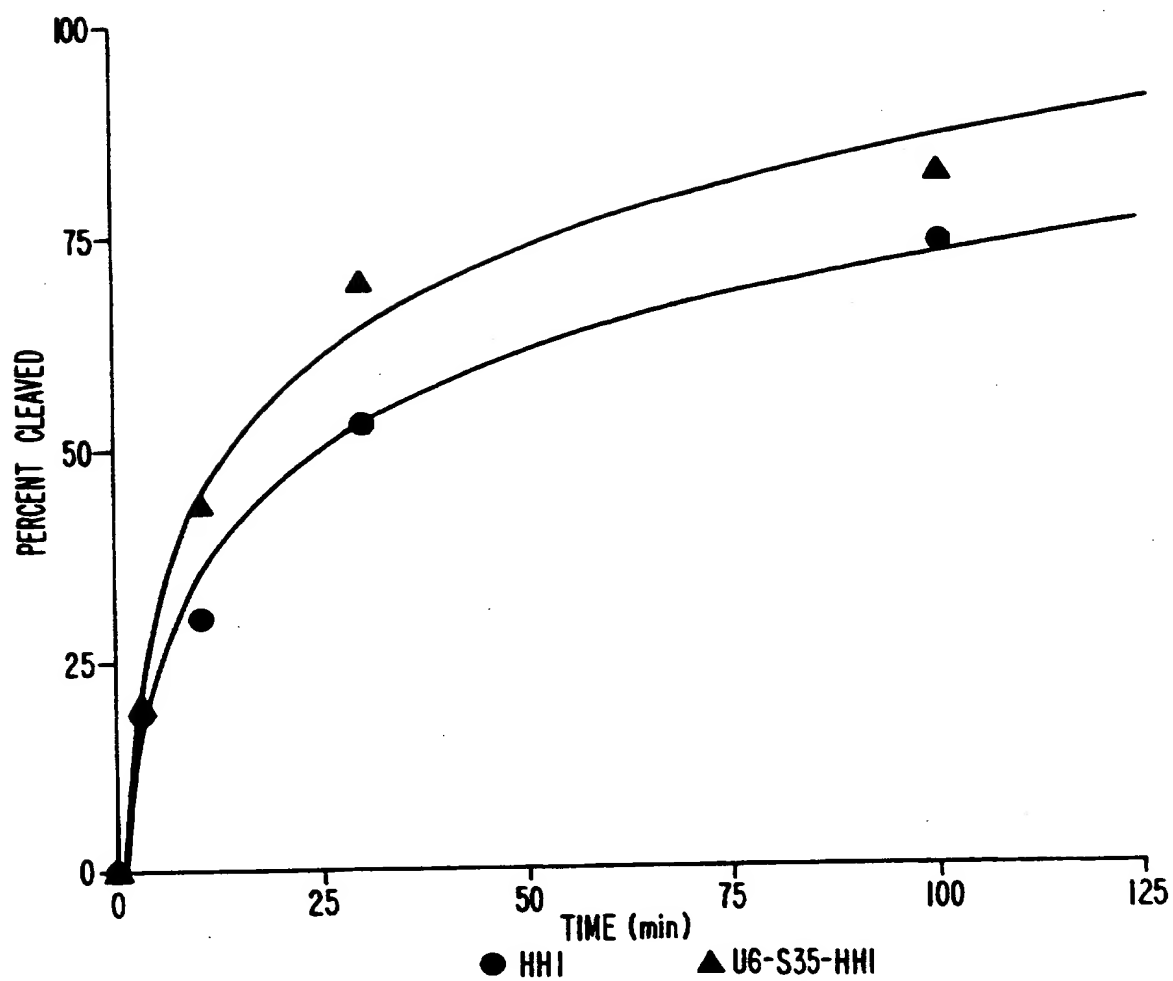


FIG. 80.

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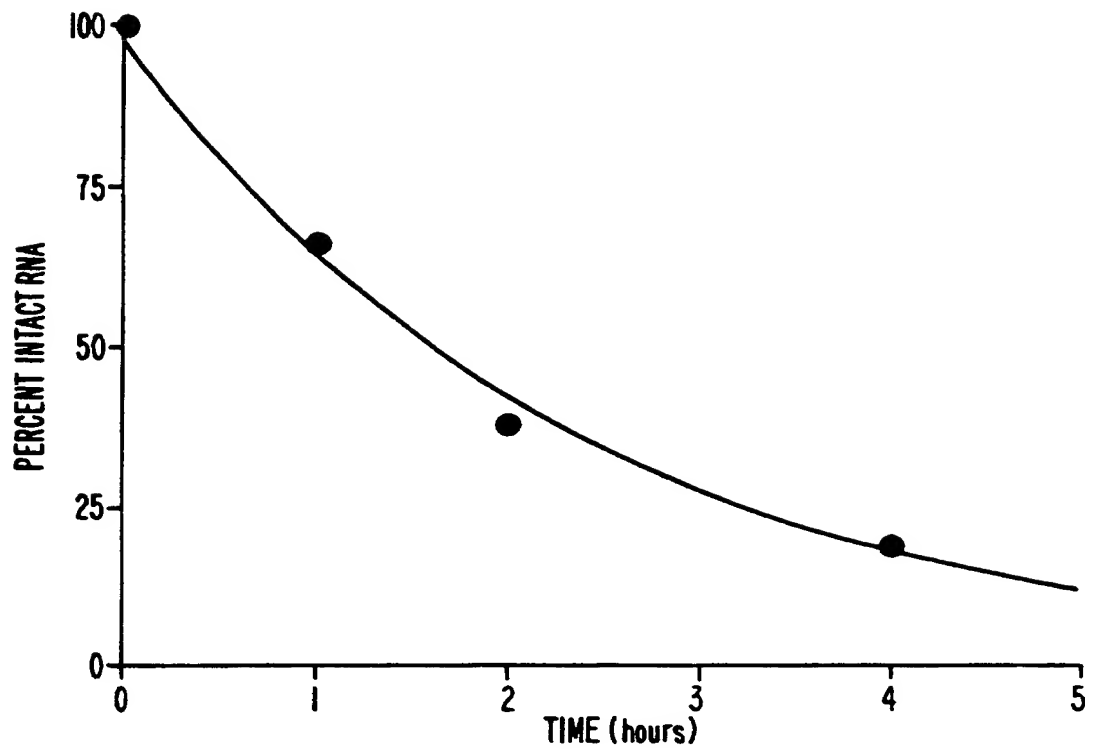


FIG. 81.

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FIG. 82.

Apical Stem-loop

Central Domain

Terminal Stem

5P G-CCUUU n
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VA1-Chimera

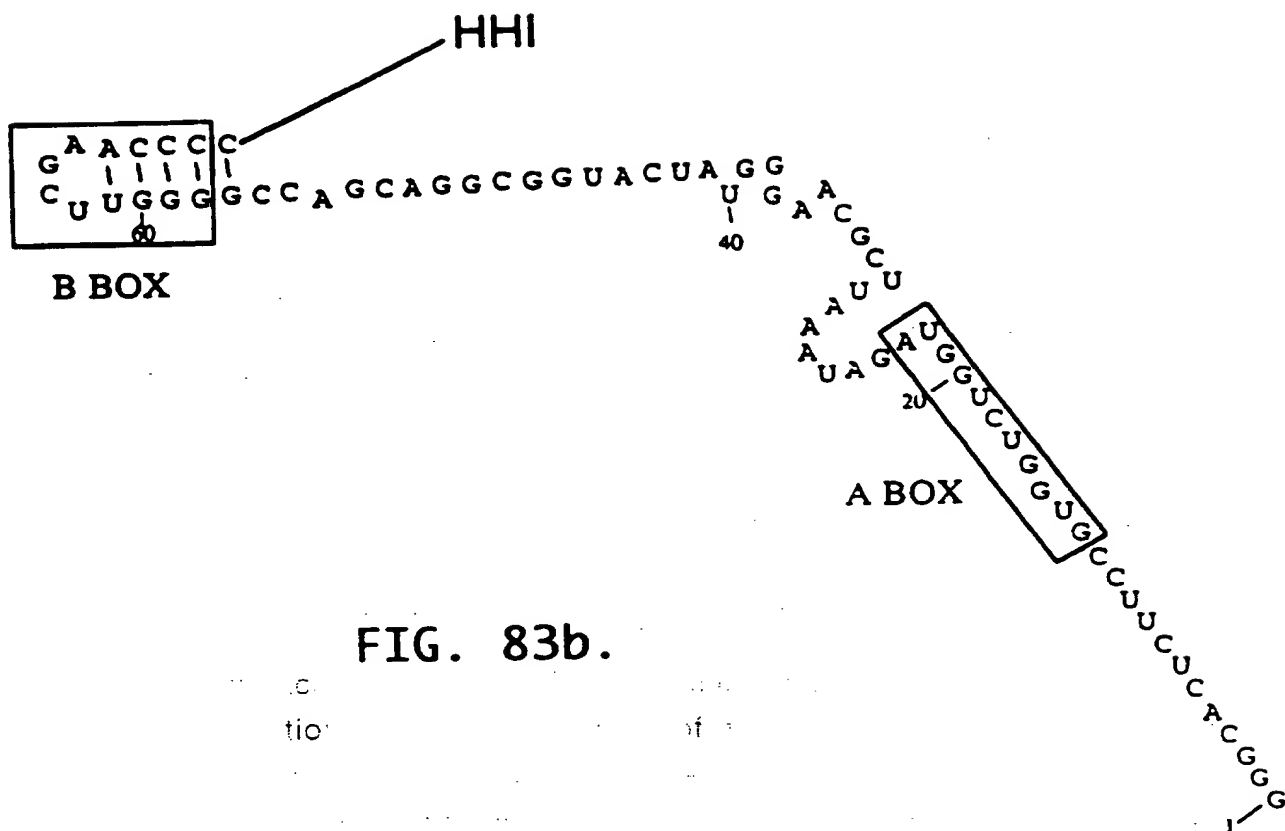


FIG. 83b.

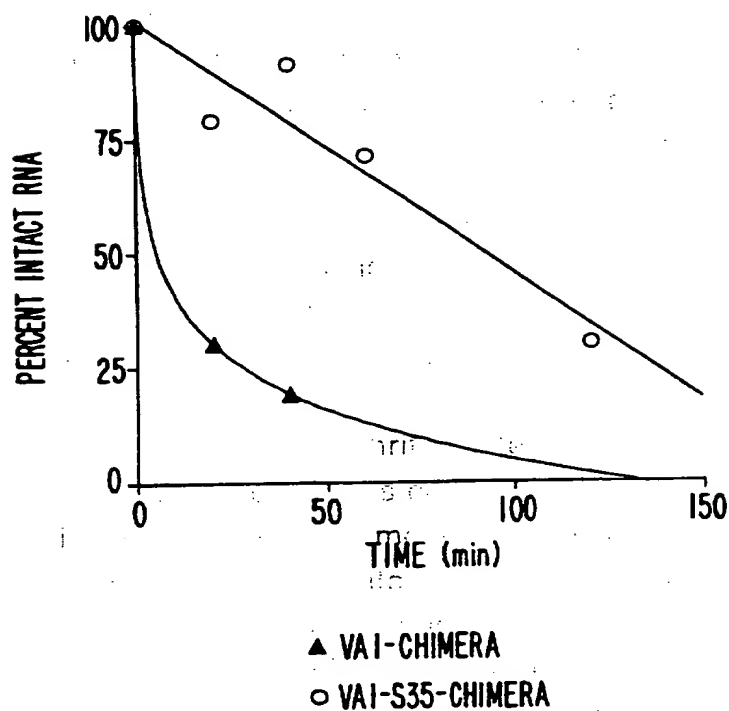


FIG. 84.

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BNSDOCID: <WO_____9618736A3 ! >

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/52 C12N9/00 C07H21/00 C07H19/04 C07H19/10
 C07H19/20 A61K31/70 C12N15/85 C12N15/86 C12N5/10
 //C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07H A61K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NUCL.ACIDS SYMP.SER. 31 (09/11-11-94);163-4, XP002002020 USMAN, N. ET AL. 'Chemical modification of hammerhead ribozymes: activity and nuclease resistance' cited in the application	1,2
Y	see the whole document	7 d
Y	ERICKSON, R. & IZANT, J. 'GENE REGULATION: BIOLOGY OF ANTISENSE RNA AND DNA'; RAVEN PRESS, NEW YORK, US; 1992 pages 35 - 54, XP002002021 TAIRA, K. & NISHIKAWA, S. 'Construction of several kinds of ribozymes: their reactivities and utilities' see page 44, last paragraph - page 46, paragraph 1	7

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *A* document member of the same patent family

Date of the actual completion of the international search

3 May 1996

Date of mailing of the international search report

07.08.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax (+31-70) 340-3016

Authorized officer

ANDRES S.M.

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO,A,94 02595 (RIBOZYME PHARMACEUTICALS INC) 3 February 1994 cited in the application see page 30</p> <p>---</p> <p>CURRENT OPINION IN STRUCTURAL BIOLOGY, vol.4, no.3, 1 June 1994 pages 322 - 330, XP000523747 SYMONS R H 'RIBOZYMES'</p> <p>---</p> <p>J.EXP.MED. 180 (1994),631-40, XP000568831</p> <p>---</p> <p>WO,A,95 23225</p> <p>---</p> <p>WO,A,93 23569</p> <p>---</p> <p>WO,A,95 04818</p> <p>---</p> <p>WO,A,95 13378</p> <p>---</p> <p>WO,A,95 13380</p> <p>---</p> <p>BBA 1219 (1994),405-12 [ZP303]</p> <p>---</p> <p>NAR 23 (1995),2677-84</p> <p>---</p> <p>JBC 270 (1995), 25702-....</p> <p>---</p> <p>EP,A,0 519 463</p> <p>---</p> <p>WO,A,91 10671</p> <p>-----</p>	1,2,7

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 63
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The attention of the applicant is drawn to the fact that claim 63 is missing.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

For further information please see enclosed form!

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1,2 and partially 7.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

THE APPLICATION CONTAINS THE FOLLOWING INVENTIONS:

- 1) Claims 1-2 (totally) and 7 (partially)

An enzymatic nucleic acid having a hammerhead motif comprising at least 5 ribose residues, at least 10 2'-O-methyl modifications, a 3'-end modification and a 2'-C-allyl modification at position 4, optionally further modified in 5' with phosphorothioate linkages.

- 2) Claims 3 (totally) and 7 (partially)

An enzymatic nucleic acid having a hammerhead motif comprising at least 5 ribose residues, at least 10 2'-O-methyl modifications, a 3'-end modification and a 2'-amino modification at positions 4 and/or 7, optionally further modified in 5' with phosphorothioate linkages.

- 3) Claims 4,64-68 (totally) and 7 (partially)

An enzymatic nucleic acid having a hammerhead motif comprising at least one non-nucleotide substitution, optionally further modified in 5' with phosphorothioate linkages, and a mammalian cell comprising such a nucleic acid.

- 4) Claims 5-6 (totally) and 7 (partially)

An enzymatic nucleic acid which cleaves stromelysin mRNA, optionally comprising phosphorothioate linkages at its 5'-end.

- 5) Claims 8-29 (all partially)

An enzymatic nucleic acid which blocks synthesis and/or expression of an mRNA encoding B7-1, cells containing it, an expression vector encoding it, a cell containing this vector, its use in the treatment of conditions associated with the level of B7-1, in a method for inducing tolerance to an alloantigen, for enhancing graft tolerance or for treatment of an autoimmune disease.

- 6) Claims 8-29 (all partially)

An enzymatic nucleic acid which blocks synthesis and/or expression of an mRNA encoding B7-2, cells containing it, an expression vector encoding it, a cell containing this vector, its use in the treatment of conditions associated with the level of B7-2, in a method for inducing tolerance to an alloantigen, for enhancing graft tolerance or for treatment of an autoimmune disease.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

- 7) Claims 8-29 (all partially)

An enzymatic nucleic acid which blocks synthesis and/or expression of an mRNA encoding B7-3, cells containing it, an expression vector encoding it, a cell containing this vector, its use in the treatment of conditions associated with the level of B7-3, in a method for inducing tolerance to an alloantigen, for enhancing graft tolerance or for treatment of an autoimmune disease.

- 8) Claims 8-29 (all partially)

An enzymatic nucleic acid which blocks synthesis and/or expression of an mRNA encoding CD40, cells containing it, an expression vector encoding it, a cell containing this vector, its use in the treatment of conditions associated with the level of CD40, in a method for inducing tolerance to an alloantigen, for enhancing graft tolerance or for treatment of an autoimmune disease.

- 9) Claims 30-43

An enzymatic nucleic acid having at least one modified base substitution, and a mammalian cell comprising it.

- 10) Claims 44-49

2'-deoxy-2'-alkyl nucleotide, nucleoside, or nucleotide triphosphate, oligonucleotide or enzymatic nucleic acid containing such a nucleotide, and a method for producing this enzymatic nucleic acid.

- 11) Claim 50

A method for synthesis of 2'-C-allyl derivative from a 5'-O-DMT-3'-O-TBDMS-base.

- 12) Claims 51-62

A compound having a 2'-O-alkylthioalkyl or a 2'-C-alkylthioalkyl modification, oligonucleotide or enzymatic nucleic acid containing it, cells comprising that compound or that enzymatic nucleic acid, and a method for producing such an enzymatic nucleic acid.

- 13) Claim 69

Method for synthesis of abasic ribonucleoside mimetics.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

- 14) Claims 70-74

Methods for deprotection of RNA

- 15) Claims 75-102

Method for synthesis of an enzymatic nucleic acid by coupling a 3' and a 5'-portion of said enzymatic nucleic acid with chemically reactive groups, and mixtures comprising said 3' and 5'-portions.

- 16) Claims 103-108

A transcribed non-naturally occurring RNA molecule comprising a desired therapeutic RNA portion.

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